THE UNIVERSITY OF ALBERTA

REPRODUCTIVE BIOLOGY AND DEVELOPMENT OF THE GOOSENECK BARNACLE, *POLLICIPES POLYMERUS*, WITH SPECIAL EMPHASIS ON PERISTALTIC CONSTRICTIONS IN THE FERTILIZED EGG

by

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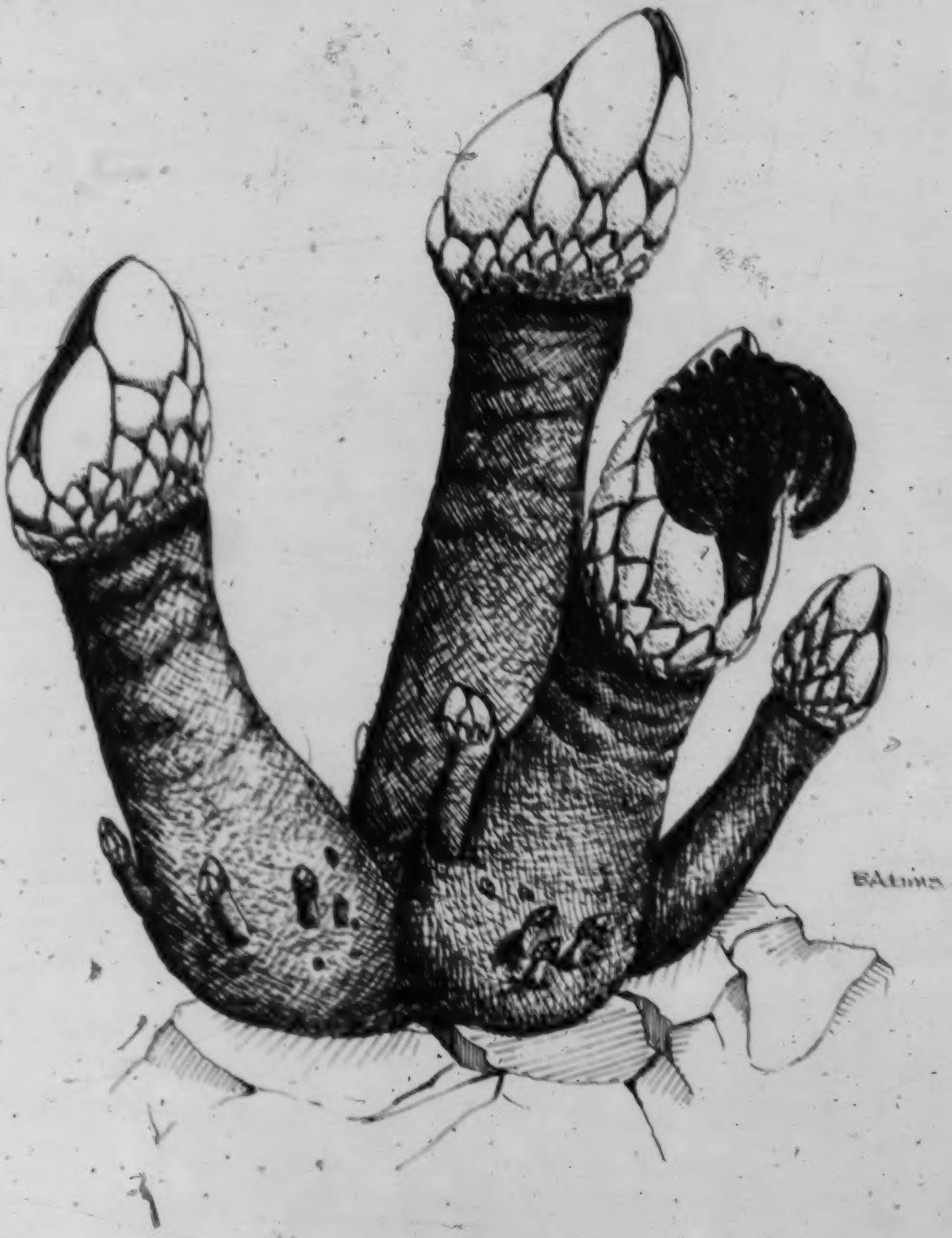
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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled REPRODUCTIVE BIOLOGY AND DEVELOPMENT OF THE GOOSENECK BARNACLE, *POLLICIPES POLYMERUS*, WITH SPECIAL EMPHASIS ON PERISTALTIC CONSTRICTIONS IN THE FERTILIZED EGG, submitted by Cynthia L. Arey Lewis in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

This dissertation concerns various aspects of growth, reproduction and development in *Pollicipes polymerus*, the gooseneck barnacle.

Four populations (2 localities and 2 intertidal levels) from San Juan Island, Washington, were sampled monthly for a total period of 26 months for the comparison of brooding cycles and fecundity. The size at reproductive maturity, mode of fertilization and probable stimulation of copulation are described.

Juveniles exhibit a cirral beating rhythm similar to that described for most operculate barnacles, whereas adults leave their cirri extended during feeding. It is suggested that the cirral beating probably involves feeding. When juveniles have increased in size to about 14 mm and large organic material becomes a greater component of the diet, the cirral beating stops.

Adults orient their cirral nets mostly toward the backwash of waves, but animals not on the backwash side of a large group tend to orient away from the center of the cluster. The degree of such orientation depends on 1) cluster size and 2) topography of the substratum where the cluster is located. A model is proposed to illustrate the adaptive significance of such mixed orientation.

The effects of several conditions on embryonic and larval cultures are reported. Embryo growth rate is accelerated under conditions closely simulating those in the adult barnacle mantle cavity (e.g., darkness and aeration). Reduction of egg mass size and addition of antimicrobial drugs also promotes fast growth. Interactions between algal species may affect their nutritional value for nauplii. Animals given small amounts of food have slower growth rates.

Development from fertilization through settlement is described. *In vitro* fertilization was accomplished using sperm already implanted in the mantle cavity plus ovulating eggs and oviducal gland fluid. The small, but yolky eggs display total, asynchronous and unequal cleavage. Division of the D macromere lags behind division of the other blastomeres, and gastrulation occurs by epiboly. The nauplii hatch 20 to 30 days after fertilization. As nauplii molt, the number of setae per appendage increases. In contrast to other pedunculate larvae, the nauplii are small and have specialized setae. Healthy adult peduncles are strongly preferred by cypris larvae as a settling substrate.

In fertilized eggs peristaltic constriction rings similar to contractile rings of cleaving cells and polar lobes move unidirectionally from the animal to the vegetal pole. In electron micrographs thin microfilaments are in the egg cortex only where there are constriction rings. Filaments are oriented primarily in meshworks, although circumferentially oriented microfilaments are also observed in vegetal rings. Microvilli extend into the space created between a constriction and the egg membrane. Subcortically, large numbers of multivesiculate bodies, glycogen granules and mitochondria are observed at the level of constriction rings. A model is proposed to explain the peristalsis. It is suggested that the function of peristaltic constriction rings is elongation of the egg from a sphere to an ovoid, although other possibilities such as lifting of the egg membrane, segregation of the lipid yolk, and predetermination of the first cleavage plane can not be ruled out.

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TABLE OF CONTENTS

Page

ABSTRACT iv
ACKNOWLEDGMENTS vi
LIST OF TABLES xiii
LIST OF FIGURES xv

Chapter

I. GENERAL INTRODUCTION 1
II. LITERATURE REVIEW 4
  Feeding 4
  Growth Rate Studies 5
  Reproductive Systems 6
  Gametes 7
  Insemination 8
  Exogenous and Endogenous Factors and Reproduction 9
  Fecundity 10
  Descriptive Embryology 11
  Factors Influencing Embryogenesis 12
  Hatching 13
  Larval Development and Settlement 13
  Peristaltic Constrictions in the Fertilized Egg 15
III. STUDY SITES 16

Chapter Page

IV. FEEDING STRATEGY 22

Introduction 22

Materials and Methods 23

Observations and Results 24

Cirral Beating 24

Gut Content Analysis 25

Experimental Stimulation of Feeding 31

Cirral Orientation 31

Discussion 42

V. GROWTH AND REPRODUCTIVE BIOLOGY 47

Introduction 47

Materials and Methods 48

Growth 48

Reproductive Biology 48

Results 52

Growth 52

Reproductive Biology 59

Discussion 84

Growth 84

Reproductive Biology 85

VI. SOME OBSERVATIONS ON FACTORS AFFECTING EMBRYONIC AND LARVAL GROWTH *IN VITRO* 103

Introduction 103

Chapter Page

VI. (Cont'd)

Materials and Methods 104
Culture of Embryos 104
Culture of Larvae 105
Preliminary Screening of Foods and Culture Conditions 106
Food Type and Concentration Experiments 107
Results 110
Factors Affecting Embryonic Development *in vitro* 110
Effects of Food Type on Larval Development 114
Effects of Larvae-to-Food Ratios on Larval Development 122
Discussion 129
Embryonic Growth *in vitro* 129
Naupliar Growth *in vitro* 133
VII. DEVELOPMENT: FERTILIZATION THROUGH SETTLEMENT 136
Introduction 136
Materials and Methods 137
Fertilization Events 137
Culture of Embryos and Larvae 137
Description of Larvae 138
Settlement of Cyprids 138
Results 139
Embryogenesis 139
Hatching and Early Nauplius Stages 146
Description of Larval Stages 146

Chapter Page

VII. (Cont'd)

Discussion 163

VIII. PERISTALTIC CONSTRICTIONS IN THE FERTILIZED EGG 169

Introduction 169

Materials and Methods 171

Observations and Results 174

Stages of Constriction 174

Cine Film Analysis 174

Effect of Some Chemical Inhibitors 181

Peristaltic Constriction Rings in Temporal Perspective 182

Fine Structure of Constriction Ring Stage: General Features 193

Fine Structure of Constriction Rings 202

Effects of Cytochalasin B 223

Effects of the Dimethylsulfoxide Control (DMSO) 228

Effects of Antimycin A 228

Discussion 228

Organelles in the Peristaltic Constriction Ring Model 242

Possible Mechanisms for Propagating Peristaltic Constriction Rings 248

Possible Function of Peristaltic Constriction Rings 250

IX. GENERAL DISCUSSION AND CONCLUSIONS 253

Environmental Effects 253

Developmental Events 269

Page
LITERATURE CITED 278

APPENDIX I.

COMPUTER PROGRAM CALCULATING TOTAL DAILY IMMERSION TIMES AND DATA 309

LIST OF TABLES

Table Page

1 Midgut contents of *Pollicipes polymerus* 30

2 Identifiable food items in order of frequency found in the midgut of the gooseneck barnacle 32

3 Reproductive condition of *Pollicipes polymerus* collected at 4 sites 75

4 Gooseneck barnacles brooding embryos at 2 localities over a 26 month period 78

5 Species found within clusters of *Pollicipes polymerus* at 2 study localities 79

6 Mean dry weight of *Pollicipes polymerus* embryo masses at 4 study localities 82

7 Fecundity in 4 populations of *Pollicipes polymerus* . . . 83

8 Breeding periods of various cirripedes 88

9 Fecundity of *Pollicipes polymerus* at different latitudes 98

10 Results of naupliar diets 108

11 Growth time from nauplius stage 2 through 6 109

12 Efficacy of several algal species as food for *Pollicipes polymerus* 111

13 Embryonic development time from formation of the labrum to naupliar hatching 113

14 Naupliar growth statistics 119

15 Growth time from hatching to naupliar stages 120

16 Embryonic developmental timetable for *Pollicipes* sp.: data from several species 131

17 Embryonic developmental timetable and embryo sizes of *Pollicipes polymerus* 140

Table

Page

18 Size of the nauplii of *Pollicipes polymerus* 147

19 Distribution of the abdominal process spinules in *Pollicipes polymerus* nauplii 156

20 Setation formulae of the naupliar stages of *Pollicipes polymerus* 161

21 Distribution of several organelles in *Pollicipes polymerus* eggs 224

22 Morphogenetic movements described in eggs and cells of several species 272

LIST OF FIGURES

Figure		Page
1	Map of Olympic Peninsula region of Washington State showing location of study sites	17
2	Photographs of study sites	19
3	Relationship between cirral beat rate and temperature	26
4	Relationship between cirral beat rate and animal size	28
5	Cirral orientation	34
6	Relationship of cirral orientation angle with waves and backwash	36
7	Relationship between cirral orientation, cluster size and substrate angle	38
8	Model predicting cirral orientation as a function of cluster size	40
9	Relationship between rostral-carinal length and dry weight	53
10	Growth at Edward's Reef	55
11	Growth at Eagle Point	57
12	Size frequency distribution of brooding animals	60
13	Relationship of brooding with distance from closest mature neighbor	62
14	Relationship of reproductive cycle at San Juan Island to photoperiod, and air and sea water temperature	64
15	Surface sea water temperature in Puget Sound	67
16	Embryonic stages from Eagle Point	69
17	Embryonic stages from Edward's Reef	71
18	Percent of individuals brooding in 4 populations on San Juan Island	76

Figure Page

19 Growth of naupliar length on several diets 115

20 Growth of nauplii from stage to stage on several diets 117

21 Effect of diet on naupliar growth 123

22 Growth of naupliar length and width in larvae eating 2 densities of *Prorocentrum micans* 125

23 Growth of nauplii from stage to stage in larvae eating 2 densities of *Prorocentrum micans* 127

24 Embryonic development: fertilization through gastrulation 142

25 Embryonic development: division of yolky cells through hatching 144

26 Outlines of the 6 naupliar stages 149

27 Labra of the 6 naupliar stages 151

28 Abdomens of the 6 naupliar stages 154

29 Antennules and mandibles of the 6 naupliar stages 157

30 Antennae of the 6 naupliar stages 159

31 Drawing of the cypris larva 164

32 Constriction rings in newly-fertilized egg from a time-lapse movie sequence 175

33 Peristaltic movement of constriction rings in different stages 177

34 Relationship of amplitude of constriction with position of constriction along animal-vegetal axis and stage 179

FIGURES 35 to 56: An Ultrastructural Study of Peristaltic Constriction Rings in *Pollicipes polymerus*.

35 Light and electron micrographs of the first polar body 184

36 Fine structure of the fertilized egg at the first polar body stage 186

Figure Page

37 Scanning and transmission electron micrographs of eggs with constriction rings 189

38 Scanning and transmission electron micrographs of eggs with a second polar body. 191

39 Nuclear material after second polar body formation 194

40 Diagram illustrating 3 planes of sectioning through an egg with peristaltic constriction rings . . . 196

41 Transmission electron micrographs from 3 different planes of section in an egg with peristaltic constriction rings 198

42 Extracellular material associated with egg during constriction 200

43 Yolk vesicularization, reversed micropinocytosis, and mitochondrial activity 203

44 Yolk vesicularization through release of vesicles by multivesiculate bodies 205

45 Reversed micropinocytosis and yolk breakdown 207

46 Longitudinal views of the animal pole, middle-position constriction ring, and vegetal constriction ring 210

47 Grazing longitudinal section of a vegetal constriction ring 212

48 Equatorial sections between constriction rings and through a constriction ring 214

49 Microfilamentous meshwork in a vegetal constriction ring 217

50 Grazing longitudinal sections of vegetal constriction rings with equatorial microfilaments 219

51 Equatorial section of a vegetal constriction ring and longitudinal section of an egg treated with CCB . . . 221

52 Eggs treated with CCB, with amorphous material, microtubules, myelin body and vacuoles 226

53 Eggs treated with dimethylsulfoxide, antimycin A, and ethanol 229

Figure Page

54 Diagram of eggs showing 4 possible arrangements of microfilaments 234

55 Diagram of a spherical egg and one with a single constriction showing probable changes in the surface 236

56 Model of the peristaltic constriction event 245

57 Average adult size at 2 intertidal heights at Eagle Point and Edward's Reef 255

58 Juvenile growth rate at 2 intertidal heights at Eagle Point and Edward's Reef 257

59 Percent brooding in an average month at 2 intertidal heights at Eagle Point and Edward's Reef 259

60 Mean monthly egg production at 2 intertidal heights at Eagle Point and Edward's Reef 261

61 Variation in sea water temperature and reproductive period with latitude 267

62 Total daily immersion times during the reproductive period at Edward's Reef 317

63 Total daily immersion times during the reproductive period at Eagle Point 319

GENERAL INTRODUCTION

Pollicipes polymerus Sowerby 1833 is one of the most common intertidal invertebrates of the unprotected rocky shore on the West Coast of North America. Most of the literature on various aspects of the biology of this species has been published within the past 20 years: location and characterization of neurosecretory cells (Barnes and Gonor, 1958); feeding behavior and morphology of the feeding apparatus (Barnes and Reese, 1959; Howard and Scott, 1959); general behavior of the animal as related to its ecology (Barnes and Reese, 1960); analysis of fatty acids in lipid fractions of adults (Rodegker and Nevenzel, 1964); characterization of some intermediary metabolic pathways in the developing embryos (Eastman, 1968); study of the animal's carotenoid pigments (Holter, 1969); morphology of the circulatory system and speculation as to the function of its parts (Burnett, 1972); identification of the animal's tolerance to temperature changes, desiccation and osmotic stress (Fyhn *et al.*, 1972); respiratory adaptations (Petersen *et al.*, 1974); reproductive cycles at Monterey Bay, California (Hilgard, 1960); the effect of oil pollution on breeding at Santa Barbara, California (Straughan, 1971) and San Francisco, California (Hand *et al.*, 1973); and study of synaptonemal complexes in spermatocytes (Dudley, 1973).

The distribution of this species is given as the Bering Straits, Siberia (found on a humpback whale in 1865) to Rosario, Baja California (Pilsbry, 1907). Pilsbry (1907) observed that the next most northern location is near Susk, British Columbia. Tarasov and Zevina (1957) recorded this species from Sakalin Island in the Western Pacific.

P. polymerus has been described as an exclusively littoral species

(Pilsbry, 1907; Barnes and Reese, 1960) found in the upper two-thirds of the intertidal zone, commonly associated with *Mytilus californianus* and *Pisaster ochraceus* along the open California coast in areas with strong wave action (Cornwall, 1955a,b). Barnes and Reese (1960) thought that the San Juan Islands present an exception to the animal's normal habitat, since it is supposedly more protected here than on the outer coast. Rice (1930), Towler (1930), Henry (1940a,b) and Barnes and Reese (1960) observed the species above low tide, extending to higher levels in crevices than on horizontal substrate in the islands. It has been reported that *P. polymerus* is found only in dense clusters and is rarely solitary (Rice, 1930; Shelford, 1930; Towler, 1930; Cornwall, 1955a; Barnes and Reese, 1960). Barnes and Reese (1960) also observed that the form of the cluster depends largely upon the degree of wave exposure and the microenvironment.

Although the objectives have been enumerated in each chapter, they are summarized as follows:

1) to culture the embryos and larvae up to settling, as this has not been done previously;

2) to carry out a fine structural analysis of eggs displaying peristaltic constriction, a very important morphogenetic event which has been only incidentally observed in passing (e.g., Nussbaum, 1890);

3) to study the reproductive cycle and fecundity on San Juan Island, Washington, and to compare these data with what is known (Hilgard, 1960; Straughan, 1971; Hand *et al.*, 1973); and

4) to examine the growth rate and feeding strategies and to compare these data with what is known (Barnes and Reese, 1959, 1960).

In this study problems of reproduction and development of *P. polymerus* have been approached in 3 different ways:

1) a field study of growth, reproductive cycles and fecundity,

2) a laboratory investigation of embryonic and larval development to settling, and

3) an ultrastructural examination of an early developmental event, peristaltic constriction.

The barnacle referred to as *Pollicipes* in this dissertation was described in 1815 by Oken as *Mitella* (see Darwin, 1851), but the generic name was changed in 1959 by the International Commission on Zoological Nomenclature to *Pollicipes* (Leach) on the basis of common usage (W. Newman, Personal communication). Sowerby described *Pollicipes polymerus* in 1833.

LITERATURE REVIEW

Until 1830 the phylogenetic position of barnacles had puzzled biologists. Earliest accounts recall geese borne by stalked barnacles, hence their present name "gooseneck barnacles" (Cornwall, 1955a). Linnaeus relegated all barnacles to the generic name *Lepas* and placed them with the molluscs. In 1817 Cuvier placed them between molluscs and annelids (Darwin, 1851), while Saint Ange (1835) insisted that they were related to annelids. Strauss (1819) first postulated the relationship of barnacles to Crustacea, but his observations were disregarded until Thompson (1830), an amateur naturalist, published his record of cypris larvae metamorphosing into acorn barnacles (observations in the spring, 1826) and this was later confirmed by Gray (1833) who described the nauplii. Since then, monographs and descriptive works on the Cirripedia have begun to answer many of the taxonomic questions (Darwin, 1851, 1854; Gruvel, 1905; Pilsbry, 1907, 1916, 1921; Nilsson-Cantell, 1921; Broch, 1922; Cornwall, 1925, 1951, 1955a,b; Henry, 1940a,b).

Feeding

The rhythmic cirral activity of many barnacles is well known as a mechanism for the capture of food (Darwin, 1854). It has been shown that in most species of operculate barnacles, any individual can display several types of activity (Southward, 1955b) which differ not only in rate of beating, but also in the degree of muscular activity developed in each beat and the resulting mechanism of feeding (Southward and Crisp, 1958).

Various types of cirral activity have now been described (Crisp and Southward, 1961). The presence of a water current in the mantle cavity (Nilsson-Cantell, 1921) and the possible occurrence of macrophagy (Barnes, 1959a) have been linked to the numerous descriptions of captorial feeding on large particles (Darwin, 1854; Gruvel, 1905; Batham, 1945; Barnes and Reese, 1959; Howard and Scott, 1959). In addition, Barnes and Reese (1959) have described the structure of the cirri and mouthparts and the probable method of feeding of *Pollicipes polymerus*.

Crisp and Stubbings (1957) first described the orientation of barnacles in relation to the direction and force of water currents. Subsequently, Barnes and Reese (1959) suggested that *P. polymerus* orients with its cirral net facing the backwash of waves as the water pours over rocks and boulders.

Growth Rate Studies

With a knowledge of the size to age relationship and the growth rate *in situ*, the age of field animals may be determined, and the age at sexual maturity and the length of the life span may be subsequently calculated. All other factors, however, must remain constant. Broch (1922) reported that the so-called "lines of growth" on the plates of *P. polymerus* are irregular, bearing no apparent connection with the environment. Since the number of lines differs in various plates on 1 animal, they are apparently unsuitable for use in aging.

Most barnacles are reported to be fast growers with short life spans such as 1 to 2 years (Darwin, 1851, 1854; Gruvel, 1905; Runnstrom, 1925; Moore, 1934a; van Breemen, 1934; Crisp and Chipperfield, 1948; Bousfield, 1952-53; Costlow and Bookhout, 1953, 1957; Blom, 1965; Werner, 1967), including some pedunculates (Evans, 1958; Skerman, 1958).

However, Batham (1945) showed that *P. spinosus* grows slowly and evidence points to the same for *P. polymerus* (Barnes and Reese, 1960). Growth is affected by: 1) amount and type of food (Moore, 1935b; Crisp, 1950; Barnes and Barnes, 1954), 2) breeding (Crisp, 1960; Crisp and Patel, 1961), 3) temperature (Coe, 1932; Moore, 1935a; Zenkewitch, 1935; Southward, 1950; Barnes and Barnes, 1954, 1956b), 4) salinity (John, 1964; Sandison, 1966), 5) position in relation to the rest of the population (Crisp and Davies, 1955), 6) population density (Moore, 1935b), 7) water flow rate (Moore, 1935b; Crisp and Davies, 1955; Crisp, 1960; Crisp and Patel, 1961; Werner, 1967) and 8) intertidal level (Moore, 1935b; Hatton, 1938; Barnes and Powell, 1953; Barnes and Barnes, 1956b; Luckens, 1968) in some barnacle species.

Reproductive Systems

Although many workers have considered a number of aspects of barnacle reproduction, only 4 (Nussbaum, 1890; Hilgard, 1960; Straughan, 1971; Hand *et al.*, 1973) have treated *P. polymerus*. Since, in a number of respects, all free living barnacles (e.g., acorn and stalked) have comparable reproductive strategies, a summary of previous work is presented here.

Saint Ange (1835) first described the male reproductive system, followed by Nussbaum (1890) and Gruvel (1893). Wagner (1834) and Saint Ange (1835) recognized the ovaries. However, Goodsir (1843) confirmed Cuvier's erroneous description of male organs as female organs. Darwin (1851) also misunderstood the female system, but correctly reported the male system. Krohn (1859) first described the female reproductive system of *Lepas anatifera* correctly, and this was subsequently confirmed and expanded by studies of Kossman (1874), von Willemoes-Suhm (1876), Nussbaum (1890), Gruvel (1893, 1898) and Groom (1894). Batham (1945) gave a detailed histological analysis of both reproductive systems in *P. spinosus*. Crisp (1954) showed development of the testes and ovary in *Balanus porcatus* (= *B. balanus*) and their seasonal cycles. Hilgard (1960) described both reproductive systems in *P. polymerus* and showed that this species is not protandric. Crisp and Patel (1961) reported ovary and testes development in relation to animal size and breeding cycles in *Elminius modestus* grown on glass slides. Walley (1965) explained the function of the oviducal gland and described egg mass formation. Barnes and Stone (1972) observed seasonal changes in oviducal gland and penis size (secondary sexual characteristics).

Gametes

The internal structure of barnacle gametes was not studied until recently: Spence-Bate (1851) drew figures of the sperm of *B. balanoides*, *B. perforatus* and *Verruca stroemia*; von Willemoes-Suhm (1876) described *Lepas* sperm as filamentous; Nussbaum (1890) drew filiform *P. polymerus* sperm; Brown (1966), Turquier and Pochon-Masson (1969), Bocquet-Védrine and Pochon-Masson (1969) and Munn and Barnes (1970a,b) have delineated the ultrastructure of barnacle sperm. Seminal plasma has been analyzed biochemically by Barnes (1962, 1963b), Barnes and Finlayson (1962), and Barnes and Dawson (1966). Sperm activation was described by Barnes and Crisp (1956) and Walley *et al.* (1971). Only 1 ultrastructural study of oogenesis (Woods, 1969) and none of spermatogenesis has been reported to date.

Insemination

Self-fertilization was considered common to all barnacles at one time, but Darwin (1854), Müller (1867) and Gruvel (1905) presented evidence that cross-fertilization occurs frequently. It now appears that cross-fertilization is probably obligatory in *B. crenatus* (Crisp, 1950, 1954; Barnes and Crisp, 1956), *Elminius modestus* (Crisp, 1950, 1954, 1956, 1958b), *B. balanoides* (Crisp, 1950; Crisp and Patel, 1960), *B. glandula, Chthamalus dalli* (Wong, 1967), *B. balanus* (Barnes and Barnes, 1954; Crisp, 1954), and *Lepas anatifera* (Patel, 1959). However, Barnes and Crisp (1956) showed that self-fertilization can occur in *Chthamalus stellatus, Verruca stroemia* and *B. perforatus*, and Werner (1967) showed that it occurs in *B. trigonus*. Broch (1922) referred to the genus *Pollicipes* as one which cross-fertilizes, but Batham (1945) assumed that isolated adult *P. spinosus* fertilize their own eggs, and that only colonial adults cross-fertilize. Hilgard (1960) showed that *P. polymerus* isolated by 20.5 cm or more from other adults never carried embryos. However, her thesis states that there is no conclusive evidence that self-fertilization does not occur, at least occasionally (Howard, 1959). On the other hand, Barnes and Reese (1960) feel *P. polymerus* self-fertilizes most of the time, although they present no evidence in favor of this hypothesis.

Müller (1867) first described "searching" behavior of the penis of a barnacle. Crisp (1954) gave some details of mating in *B. balanus* as did Barnes and Barnes (1956a) and Clegg (1957) for *B. balanoides* and Patel (1959) for *Lepas anatifera*. Wong (1967) gave the first actual account of the insemination process and described copulatory activity and the reproductive states of the participants for *B. glandula* and *B. tintinnabulum*.

Collier *et al.* (1956) stimulated copulation in a balanoid by releasing small concentrations of ascorbic acid in the water. However, Barnes (1963a) suggested (and Crisp and Patel, 1969, concurred) that copulation in *B. balanoides* is under hormonal control. Copulation in *P. polymerus* was never witnessed in the present or in a previous study (Hilgard, 1960).

Exogenous and Endogenous Factors and Reproduction

As the effect of exogenous factors on breeding is much easier to detect than the effect of endogenous factors, most studies have emphasized the former. However, it has been shown that there are neurosecretory cells in *P. polymerus* (Barnes and Gonor, 1958) which may transfer the effect of environmental stimuli via hormonal control of gonadal development, breeding and other metabolic processes. Similar endocrine mechanisms have been postulated for crayfish (Aiken, 1969; Perryman, 1969), *B. balanoides* (Barnes, 1963a; Tighe-Ford, 1967; Crisp and Patel, 1969) and *B. balanus* (Crisp and Patel, 1969).

In many species, latitudinal differences in breeding cycles occur (Moore, 1935b; Hutchins, 1947; Bousfield, 1952-53; Barnes and Barnes, 1956b; Crisp, 1957, 1959a; Pearse, 1970). Once it has been ascertained that intraspecific differences in reproductive cycles occur latitudinally, one may be able to predict the environmental factors critical to reproduction.

Sea water temperature has been suggested as the primary influence on breeding in intertidal marine invertebrates (Appellof, 1912; Orton, 1920; Runnstrom, 1927; Hutchins, 1947; Bousfield, 1952-53), while it has been suggested that air temperature limits distribution (and therefore reproduction) in at least 1 barnacle species (Kitching, 1950;

Southward, 1950; Williams, 1950). Temperature may control several parameters of reproductive activity: 1) growing season of adequate duration for gametogenesis and inducement of the onset of spawning (Batham, 1945; Hutchins, 1947; Kitching, 1950; Bousfield, 1952-53; Crisp, 1954, 1957, 1959a; Crisp and Davies, 1955; Barnes and Barnes, 1956b, 1967; Barnes, 1957, 1959b, 1963a; Patel, 1959; Crisp and Clegg, 1960; Hilgard, 1960; Patel and Crisp, 1960a; Blom, 1965; Tighe-Ford, 1967; Crisp and Patel, 1969; Barnes and Stone, 1972), 2) length of development time (Crisp and Davies, 1955), and 3) requirement of a conditioning period (Crisp, 1959a; Crisp and Clegg, 1960; Barnes, 1963a; Crisp and Patel, 1969).

Other factors shown to influence the process of reproduction in barnacles are: 1) day length (Crisp, 1959a; Crisp and Clegg, 1960; Barnes, 1963a; Barnes and Barnes, 1967; Tighe-Ford, 1967; Crisp and Patel, 1969; Barnes and Stone, 1972), 2) intertidal height (Moore, 1935a; Crisp, 1950, 1959a,b; Luckens, 1968), 3) water currents (Moore, 1935b; Crisp, 1950, 1959a; Crisp and Davies, 1955; Crisp and Clegg, 1960), 4) food availability for gonad development and for naupliar release (Crisp and Davies, 1955; Crisp, 1957, 1959a; Patel and Crisp, 1960a; Barnes *et al.*, 1963; Barnes, 1965; Barnes and Barnes, 1967; Crisp and Patel, 1969), 5) salinity (John, 1964; Sandison, 1966), 6) lunar periodicity (Mileikovskii, 1958), 7) parasitism (Barnes, 1953), 8) pollutants (Holstrom, 1970; Straughan, 1971; Hand *et al.*, 1973), 9) individual differences (Crisp, 1959a) and 10) local topography (Crisp and Davies, 1955; Crisp, 1959a).

Fecundity

Fecundity is a scale of reproductive success which may be measured in several ways: total number, weight or volume of embryos or broods per adult per reproductive period. Various factors have been demonstrated to influence fecundity in barnacles: 1) parent size (Groom, 1894; Moore, 1935a; Batham, 1945; Kuznetzov and Matveeva, 1949; Barnes, 1953; Barnes and Barnes, 1954, 1956a; Luckens, 1968), 2) planktotrophic or lecithotrophic development (i.e., egg yolkiness) (van Hoek, 1884; Hilgard, 1960; Anderson, 1965), 3) season, temperature, weather and/or latitude (Crisp, 1954, 1959a; Crisp and Davies, 1955; Southward and Crisp, 1956; Crisp and Patel, 1969; Straughan, 1971), 4) tidal level (Moore, 1935b; Crisp, 1959a), and 5) physical factors such as oil spills (Straughan, 1971; Hand *et al.*, 1973).

Descriptive Embryology

The primary contributions to descriptive barnacle embryology up to 1902 were made by: van Beneden (1870), von Willemoes-Suhm (1876), van Hoek (1876), Lang (1878), Nassanow (1885, 1887), Nussbaum (1890) and Groom (1894). Nussbaum (1890) described development in *P. polymerus*. Not much detail was reported, but most of what was given appears to be accurate. Although most of the work up to this time was fragmentary and superficial, Groom (1894) published an extensive description primarily of late development and larval stages and divided embryonic development into stages. Many misconceptions of these early workers were clarified by the cell lineage studies of Bigelow (1902) who used both live and preserved material. He was able to orient eggs precisely before sectioning, thus aiding the pursuit of cell fates. Subsequently, Abric (1904) published a report of the origin of germ layers in *Sacculina* sp. and Delsman (1917) continued the study of cell lineage.

Batham (1946) first raised embryos continuously from 1 cell to post-cypris *in vitro* and described them histologically. Crisp (1954) outlined the embryonic stages of planktotrophic development in *B. balanus*, which agreed for the most part with Groom's (1894) results and Crisp (1959b) later confirmed this description for *B. balanoides*. Barnes (1965) developed another staging system for the same pattern of development. In 1965, Anderson described development in *Ibla quadrivalvis* and Kauffman reported specialized development in *Scalpellum* sp. Anderson (1969) also completed cell lineage studies in *Tetraclita purpurascens* and *T. rosea, Chthamalus antennatus*, and *Chamaesipho columna*. Most of this work was done with preserved material; only occasional live specimens were used.

Batham (1946) first raised embryos *in vitro*. She had the advantage, however, of working with yolky eggs. The first successful culturing of non-yolky eggs was accomplished by Crisp (1959b). He was also able to observe brooding cycles *in situ* by ingeniously inducing *B. balanoides* to settle on glass slides (a technique previously developed with *Elminius modestus*, Crisp and Davies, 1955), thus permitting observation of the embryos through the base of the adult. At this point, other culture methods were introduced with varying success (Barnes and Barnes, 1959a; Patel and Crisp, 1960b; Raffin, 1967).

Factors Influencing Embryogenesis

The major factor influencing developmental time appears to be temperature (Crisp and Davies, 1955; Crisp, 1959b; Patel, 1959; Patel and Crisp, 1960b; Barnes and Barnes, 1963; Crisp and Costlow, 1963), and temperature may also influence the interbrood interval (Crisp and Davies, 1955) and embryo size or volume (Crisp, 1954, 1960; Patel, 1959; Patel and Crisp, 1960a). Few experimental studies have been done with cultured barnacle embryos, with most concentrating on biochemical pathways and food usage during development (Dawson and Barnes, 1966; Barnes and Evens, 1967; Eastman, 1968; Woods, 1969).

Hatching

Hatching of Stage 1 nauplii from the adult mantle cavity was described in detail by Barnes (1955c). A hatching substance was shown to be present in adult tissues (Crisp, 1956; Barnes, 1957; Crisp and Spencer, 1958; Barnes and Barnes, 1959c) which stimulates naupliar activity within the egg membrane and hastens hatching. In contrast, some acrothoracian juveniles may leave the mantle cavity as "walking" cyprids (Batham and Tomlinson, 1965).

Larval Development and Settlement

Much attention has been paid to the description of larval stages, especially in attempts to differentiate between naupliar stages for the sake of elucidating life cycles and to hasten the sorting of plankton. Thompson (1830) first described the cypris larva of a balanoid and observed its metamorphosis into a settled juvenile barnacle. Later (1835), he described the release of *Lepas* sp. nauplii. Although he was the first to connect these larval stages with barnacles (thus firmly establishing their crustacean heritage), he misinterpreted the observations, thinking there were 2 major divisions: operculates with cypris larvae and pedunculates with nauplius larvae. Burmeister (1834) first recognized that *Lepas* larvae pass through both nauplius and cypris stages and Goodsir (1843) discovered the sequence through metamorphosis.

It was then accepted that Lepadidae and Balanidae have both larval types (Darwin, 1851, 1854). Early sketchy and often inaccurate descriptions of nauplii and cyprids were given by: Gray (1833), van Hoek (1876), Groom (1892, 1894), Chun (1896) of unknown species and by: Goodsir (1843), Darwin (1851), Spence-Bate (1851), Claus (1869) and Delage (1884) of known species. Larval descriptions have been published for *B. balanoides*: Goodsir (1843), Spence-Bate (1851), Groom (1894), van Hoek (1909), Bassindale (1936), Treat (1937), Pyefinch (1948a); *B. crenatus*: Herz (1833), Bassindale (1936), Pyefinch (1948a, 1949); *B. improvisus*: Munter and Buchholz (1869), Tengstrand (1931), Lucks (1940), Kuhl (1950), Buchholz (1951), Jones and Crisp (1954), Freiberger and Cologer (1966); *B. balanus*: Spence-Bate (1851), Barnes and Costlow (1961); *B. hameri*: Bousfield (1952-53); *B. tintinnabulum:* Goodsir (1843); *B. amphitrite*: Treat (1937), Costlow and Bookhout (1958), Freiberger and Cologer (1966); *B. perforatus*: Spence-Bate (1851), Groom (1894); *B. trigonus*: Freiberger and Cologer (1966); *B. eburneus*: Costlow and Bookhout (1957), Freiberger and Cologer (1966); *B. galeatus*: Treat (1937), Molenock and Gomez (1972); *B. nubulis:* Barnes and Barnes (1959b); *Chthamalus stellatus*: Spence-Bate (1851), Bassindale (1936), Moore and Kitching (1939); *Elminius modestus:* Knight-Jones and Waugh (1949); *Verruca stroemia*: Spence-Bate (1851), Bassindale (1936), Pyefinch (1948a); *Lepas* spp.: Burmeister (1834), Darwin (1851), Claus (1869), von Willemoes-Suhm (1876), Treat (1937); *Scalpellum* sp.: Darwin (1854), Lang (1878); *Sacculina* spp.: Delage (1884); *Ibla quadrivalvis*: Anderson (1965); *Pollicipes spinosus*: Batham (1946) and *Mitella (= Pollicipes) mitella*: Yasugi (1937).

Workers have often cultured barnacle nauplii in the laboratory with favorable results (Batham, 1946; Costlow and Bookhout, 1953, 1957; Moyse, 1960, 1963; Wisely, 1960; Hirano, 1962; Blom, 1965; Freiberger and Cologer, 1966; Okamoto, 1967; Tighe-Ford *et al.*, 1970).

The details of metamorphosis from cypris to settled juvenile were given by Batham (1946), Bernard and Lane (1962) and Walley (1969). The search for the stimulus of cypris larva settlement has proved long and arduous. Four major theories of settlement stimuli have been advanced: chemosensation (Mortlock, 1969; Nott and Foster, 1969; Gibson and Nott, 1971), tactile sensation (Hatton, 1938; Pomerat and Weiss, 1946; Knight-Jones, 1953; Crisp and Barnes, 1954; Barnes, 1955a, 1956), "contact chemical sensation" (Crisp and Meadows, 1962, 1963) and phototaxis (Visscher, 1928; Pomerat and Reiner, 1942; Daniel, 1957).

Peristaltic Constrictions in the Fertilized Egg

Some early invertebrate embryologists observed marked rhythmical contractions in newly-fertilized eggs of *Lepas* sp. and *Pollicipes polymerus* (Nussbaum, 1890; Groom, 1892, 1894; Bigelow, 1902; Gruvel, 1905), although Groom (1894) may have confused this event with first cleavage. This process, which has been termed "peristaltic constriction," has been studied at the light microscope level (Lewis *et al.*, 1973). No other studies of this unusual phenomenon are known.

STUDY SITES

Two study sites were chosen July, 1971, on the west side of San Juan Island where large populations of *Pollicipes polymerus* are readily available: Eagle Point (48° 27' North, 123° 2' West) and Edward's Reef (48° 30' North, 123° 8' West) (Fig. 1). The majority of the field studies were conducted at these areas.

Although the San Juan Islands lack the deep swells of the open seas, Eagle Point faces the prevailing winds and is frequently exposed to severe wave action (Dayton, 1971). It is protected only by Vancouver Island, lying 15 miles to the west. *P. polymerus* ranges from -2.0 to +6.0 feet vertically and occurs in large, rambling populations which overlap with *Mytilus californianus* in the mussel's upper distribution (Fig. 2A). At Edward's Reef, the study area consists of a large rocky surge channel, more protected than at Eagle Point due to outlying boulders in the water and high rocky walls (Fig. 2B). Fresh water runoff occurs here during most of the year, while it is limited to the rainy season at Eagle Point. *P. polymerus* is distributed from +3.0 to +8.0 feet and occurs in discrete clusters; it is too high to associate with *M. californianus* here. In both locations there are some *Thais lamellosa, T. canaliculata* and *T. emarginata* (Gastropoda) and an abundance of *Balanus cariosus* (Cirripedia), *Acmaea digitalis, A. paradigitalis* and *A. persona* (Gastropoda) occurring with *P. polymerus*. For more complete listings of species found in this community, see: Shelford and Towler, 1925; Dayton, 1971; Paine, 1974.

Figure 1. Map of Olympic Peninsula region of Washington State showing location of the 2 study sites on San Juan Island: Eagle Point (EP) and Edward's Reef (ER).

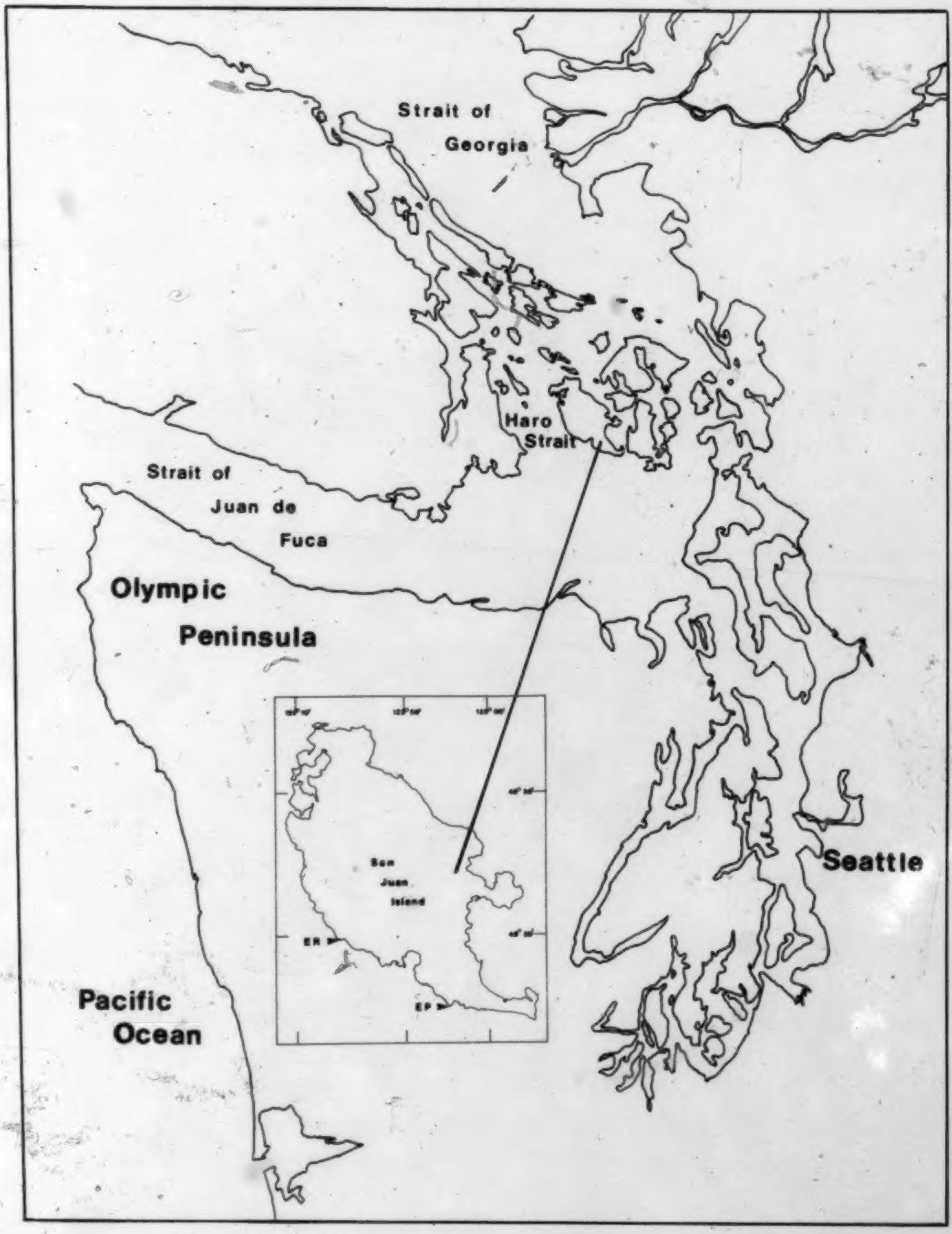

Strait of Georgia
Haro Strait
Strait of Juan de Fuca
Olympic Peninsula
Seattle
San Juan Island
ER
EP
Pacific Ocean

Figure 2. A. The study site at Eagle Point.

B. The study site at Edward's Reef.

A

B

Since one of the objectives was to evaluate the effects of wave action on communities in exposed environments, this study has been restricted to the exposed rocky intertidal localities at both study sites.

The height of a level along the shore is given as feet above or below the mean lower low water. The levels at each site were obtained by observing the low point of the low tide. This measure varied considerably from the predicted low of the tide tables due to wind and surf activity, but the reference point at each site is believed to be accurate to within ±0.5 feet. All the other levels at each site were measured in relation to the reference point with surveying equipment.

FEEDING STRATEGY

Introduction

Barnacles feed by means of cirri; the types of cirral behavior have been defined by Crisp and Southward (1961). Most observers agree that operculate species, such as *Balanus* sp., exhibit several types of active cirral movement, of which the rhythmic cirral "normal beat" is most often employed. This behavior appears to be associated with respiration and with microfeeding on suspended particulate material (Crisp and Southward, 1961; Southward and Crisp, 1965; Lockwood, 1967). In pedunculate barnacles, such as *Pollicipes* sp., however, captorial macrophagous feeding by cirral extension predominates (*P. spinosus:* Batham, 1945; *P. polymerus:* Barnes and Reese, 1959; Howard, 1959; Howard and Scott, 1959).

The barnacle orients its food capturing device, the cirral net, by twisting on its peduncle (Howard, 1959; Barnes and Reese, 1960). The adults extend their cirri for captorial feeding (Howard and Scott, 1959), and therefore depend largely on local water movements and microtopographical position for food (Barnes and Reese, 1959, 1960). It has been reported that animals in a cluster orient their cirral nets unidirectionally: perpendicular to the substrate and facing the backwash of waves (Howard, 1959; Barnes and Reese, 1960).

In this chapter evidence is presented that juvenile *Pollicipes polymerus* are, in fact, microfeeders using cirral pumping and slow cirral beating. This behavior shifts to cirral extension with increasing age. Analyses of juvenile and adult gut contents corroborate these behavioral observations. Unidirectional orientation of cirri in some small barnacle groups is confirmed, but bi- or multi-directional orientation is observed in larger groups. The mixed orientation is likely an adaptation for maximizing the feeding efficiency of each individual in the group.

Materials and Methods

Observations of juvenile beating behavior were made with animals acclimated to still or running sea water. The frequency of cirral beating was measured by recording the number of complete extensions and retractions of the cirri during 10 minutes (min) and is reported here as the number of beats per min. Ten specimens of various sizes were observed at 10° to 12°C and barnacles of a single size were observed at 10° to 15°C. The mean frequencies of beating represent the average activity of only the active barnacles. The size of animals, determined with a pair of dividers, was recorded as the distance between the base of the rostrum and the base of the carina (RC). Animals with RC measurements of 15 mm or more are defined as adults and those with less as juveniles.

For analysis of gut contents material was removed by Pasteur pipette from the midguts of frozen specimens. The material was suspended in 0.1 cm^3 distilled water and a sample was analyzed using a Neubauer hemocytometer. In case of infrequently found items, particles in 0.01 cm^3 were counted or the entire gut contents were scanned.

Natural populations of *P. polymerus* were studied at Edward's Reef. The angle of cirral orientation was measured during receding tides as waves washed over animals in surge channels. The equation of Crisp and Stubbings (1957) was employed for calculating R, the strength of orientation: $R = \sqrt{A^2 + B^2}$ where $A = 1/N \sum_i \cos \phi_i$ and $B = 1/N \sum_i \sin \phi_i$. N is the total number of individuals in the sample. When ϕ_i (angle between the RC axis and the direction towards which the current is flowing for the *i*th individual) is 0°, the cirral net faces oncoming waves; and when ϕ_i is 180°, the net faces the wave's backwash. If the population is randomly oriented, R is close to 0; if orientation is consistent throughout, R equals 1. To further quantify these observations, the clusters were rated as small (less than 35 animals), medium (35 to 150 animals) or large (more than 150 animals).

Observations and Results

Cirral Beating

In the relatively calm water of laboratory tanks, juvenile *P. polymerus* exhibit cirral beating similar to that of operculate barnacles, although slower. The extension phase of cirral beating lasts 79% of the complete beating period. In a fast current, however, these juveniles tend to extend their cirri as do adults.

As with other barnacles (Southward, 1955a), only part of the juvenile population beat their cirri at any 1 time. The percentage of individuals which are actively beating varies, depending on environmental conditions. In 1 series of observations, 44% of the juveniles beat in still sea water after they had been freshly collected, 34% beat after they had been immersed in sea water for 24 hours (h) and only 27% beat after they had been kept in air for 24 h.

The beating behavior may be partly respiratory in function for the juvenile *P. polymerus*, since when air was bubbled into the water the average rate of beat dropped slightly (from 1.4 to 1.2 per min).

If beating were purely respiratory in function, however, more animals kept in still water, away from their normal habitat of heavy surf, would be expected to have a beating or pumping activity. However, this is not the case. In addition, juvenile cirral movements resemble those in extended feeding adults, e.g., maxillae contract toward the mouth, individual cirri push food material into it, and occasional twisting and turning of capitula on peduncles occurs.

Frequency of cirral beat is also dependent upon temperature (Fig. 3), and probably reflects the general metabolic activity of the barnacle. The range of water temperatures used in this study was within normal seasonal fluctuations. Individuals were not subjected to extreme temperatures, because under such conditions cirral beat rate declines due to stress (Southward, 1955a, 1957).

At constant temperature, frequency of cirral beat was inversely correlated with size of the animals (Fig. 4). Only intermittent beating was observed in animals of 10 to 12 mm RC size, and no beating in animals of about 14 mm RC size.

Gut Content Analysis

In order to ascertain if differences in food preferences are associated with age and type of feeding behavior, analysis of gut contents was made. Although precise identification and exact numbers of individuals of each food type were difficult to obtain, the relative amounts in 3 basic food categories are presented here as percentages of total particles counted (Table 1): 1) organic particulate material, less than 10 μ in diameter, 2) inorganic particulate material, less than 10 μ in diameter, and 3) large organic material, 10 μ to 5 mm in diameter.

Figure 3. *Pollicipes polymerus*. Cirral beat rate as a function of temperature. Vertical bars represent standard deviations. All animals were 1.5 mm RC.

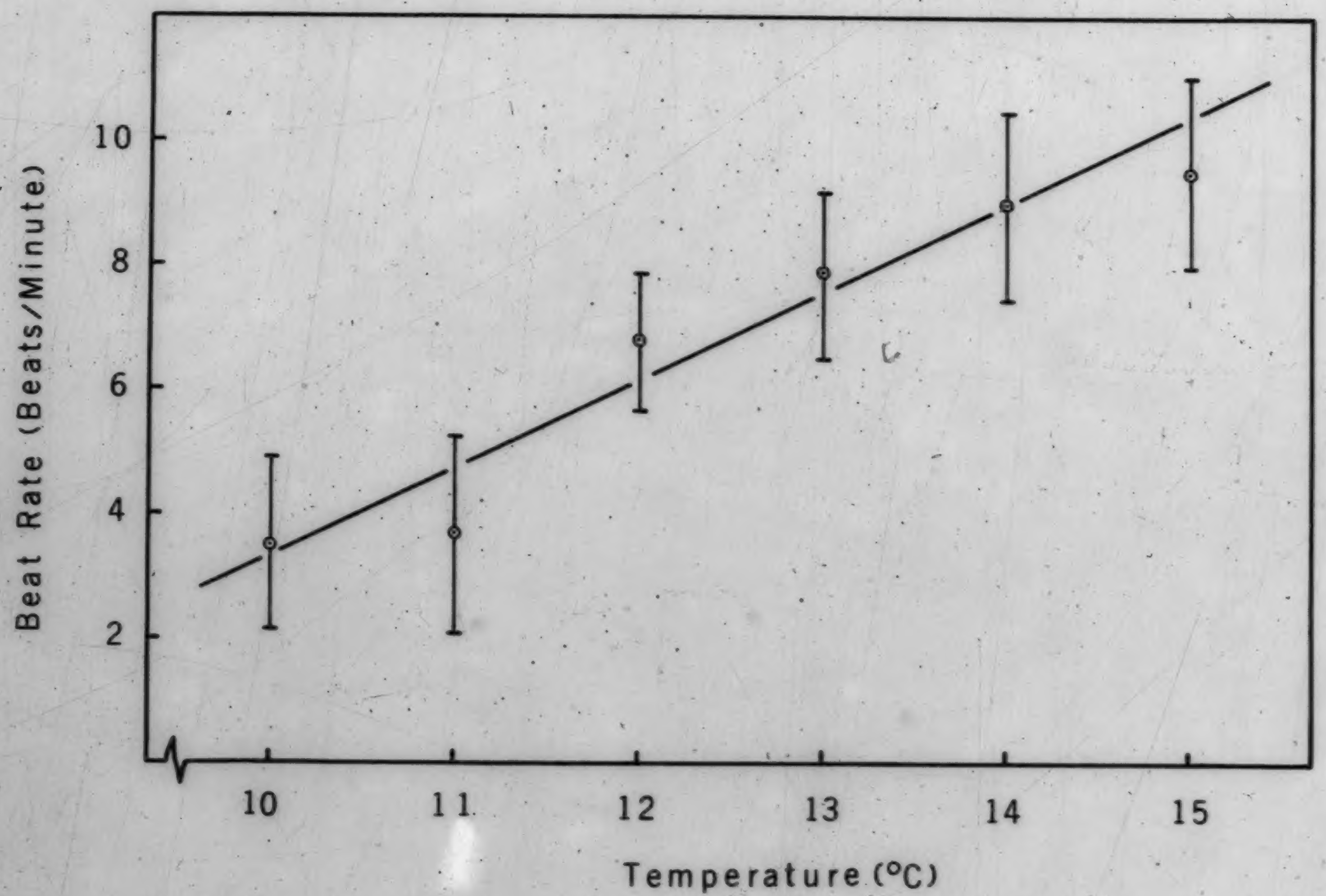

Beat Rate (Beats/Minute)
10
8
6
4
2
10
11
12
13
14
15
Temperature (°C)

Figure 4. *Pollicipes polymerus*. Cirral beat rate as a function of animal size. Vertical bars represent standard deviations. The scale is broken between 0 and 1 beat per minute to indicate intermittent beating (I). Beat rate was measured at 10° to 12°C.

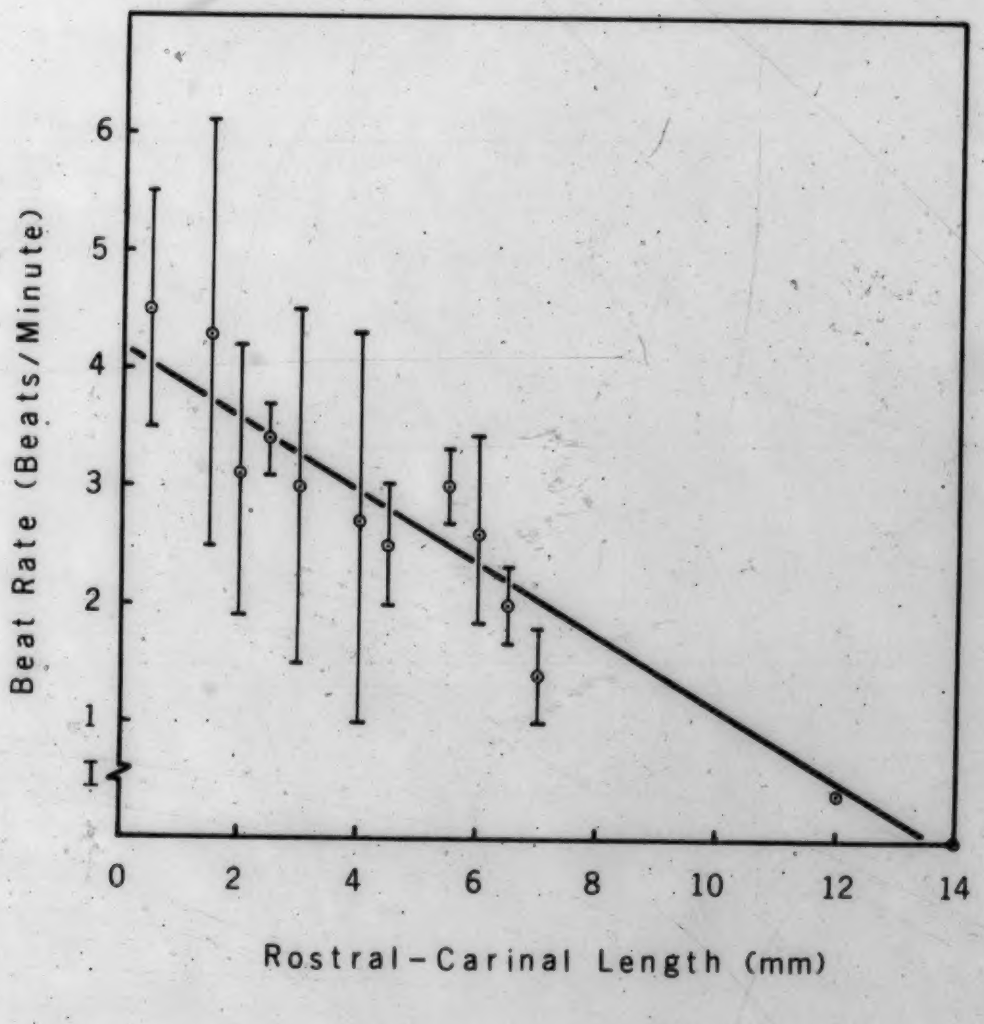

Beat Rate (Beats/Minute)
6
5
4
3
2
1
I
0
2
4
6
8
10
12
14
Rostral-Carinal Length (mm)

Table 1. *Midgut content of the gooseneck barnacle. Number of animals examined is in parentheses.*

	Animal size (mm RC)		
Percent food items	1-6 (9)	7-14 (17)	15-28 (17)
Particulate, organic	80	63	52
Particulate, inorganic	12	6	8
Large, organic	8	31	40

Table 2 lists the specific food identified and gives food preferences. As might be expected, a comparison of food particle sizes in animals of various sizes indicates that the smaller the animal, the more dependent it is on microscopic food items. Whether this reflects feeding methods, the size of the feeding apparatus, or both, remains to be clarified. The amount of inorganic material, however, varies little with animal size (see also Barnes, 1959a).

Experimental Stimulation of Feeding

Both adult and juvenile barnacles responded to the presence of introduced *Artemia salina* nauplii (up to 0.6 mm long) by voracious feeding. Adults used the typical macrofeeding habit of extension and lassoing of prey (Howard and Scott, 1959). Juveniles displayed increased beat rate, from 1.4 to 2.7 beats per min in response to food (n = 10), and often used the entire cirral net for capturing nauplii. Once the prey was captured by adults or juveniles, it was quickly moved from a cirrus or the cirral net to the mouth. When *Artemia* were first introduced to the tank, many adults exhibited intermittent pumping activity, possibly for testing the water (Crisp and Southward, 1961) before complete extension of the cirri occurred. It was apparent that *Artemia* must stimulate the inner spinous face of a cirrus before predatory behavior culminates in feeding. These observations indicate that small *P. polymerus* apparently possess the behavioral mechanisms and feeding apparatus necessary to capture large food items.

Cirral Orientation

Cirral orientation is normally bi- or multi-directional in large clusters of adult *P. polymerus*. Among the 4 small clusters studied,

Table 2. *Identifiable food items in order of frequency found in the midgut of the gooseneck barnacle. +++++ in all specimens, ++++ in most specimens, +++ in more than half of specimens, ++ in some specimens, + in few specimens, 0 in no specimens. Number of animals examined is in parentheses.*

	Animal size (mm RC)		
Food items	1-6 (9)	7-14 (17)	15-28 (17)
PARTICULATE, ORGANIC			
Detritus	+++++	+++++	+++++
Diatoms	+++++	+++++	+++++
Other unicellular phytoplankton	0	+++	++
blue-green algae	0	++	++
PARTICULATE, INORGANIC			
Shells & sand	+++	++++	++++
Sponge spicules	+	+	+
LARGE, ORGANIC			
Crustacea	+++	+++++	+++++
Barnacle exuviae	++	+++++	+++++
Copepods	0	+++++	+++++
Polychaetes	0	+++	+++
Eggs	0	+	++
Hydroids	0	+	++
Molluscs	0	+	+
Large algae	0	+	+
Echinoids	0	0	+
Shrimps	0	0	+

an average of 79% had cirri facing the local backwash (average $\phi = 156°$), while the rest faced more towards the current (average $\phi = -13^{\circ}$). Barnacles on the backwash side of the cluster face the backwash, whereas those on the wave side usually face the waves (Fig. 5). The distribution of orientation in 2 clusters is shown (Fig. 6). The majority orient toward the backwash, but a few animals orient in almost every other direction.

It was observed that the differences in the direction of cirral orientation are correlated with the size of the group. Whether or not a group displays mixed orientation seems to depend primarily on its size and to a lesser extent on the angle of the substrate (Fig. 7). The greater the number of animals in a cluster, the greater is the likelihood that the barnacles within it will show mixed orientation. Barnacle groups attached to vertical surfaces have mixed orientation slightly more often than do those in clusters of the same size attached to horizontal surfaces, possibly since vertically-oriented animals are affected more by light (Crisp and Stubbings, 1957).

A model describing cirral orientation of *P. polymerus* clusters in a semi-exposed rocky area is presented (Fig. 8). In it the cluster is depicted as oval in shape and so situated that the backwash and waves are opposed by 180° along the long axis of the cluster. In a large cluster, animals in areas A and B face the backwash, animals in area C face waves, and animals in areas D and E face away from the center of the cluster, somewhat toward the backwash. In a medium sized cluster, fewer barnacles occur in areas C, D and E, and in a small cluster, animals are limited to areas A and B. Strength of orientation (*R*) in a medium sized cluster in areas A + B is 0.95 and in area C is 0.95.

Figure 5. *Pollicipes polymerus*. Medium-sized cluster of adults showing orientation. W, wave; B, backwash.

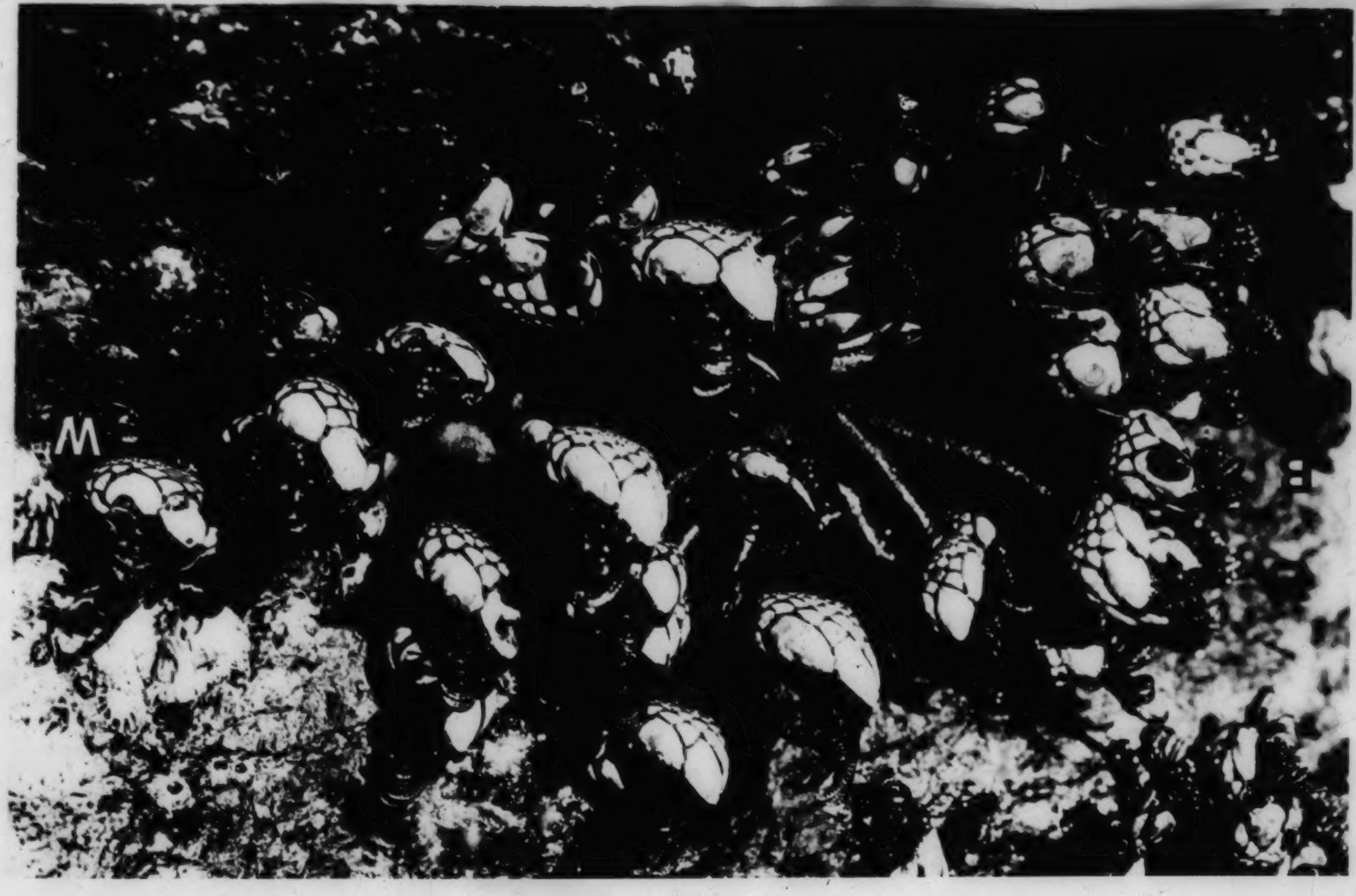

W
B

Figure 6. *Pollicipes polymerus*. Cirral orientation (ϕ) in 2 combined medium-sized clusters in a surge channel. At 0°, the cirral net points toward the waves; at 180°, cirral nets face the backwash. Frequency as defined by Crisp and Stubbings (1957) is used.

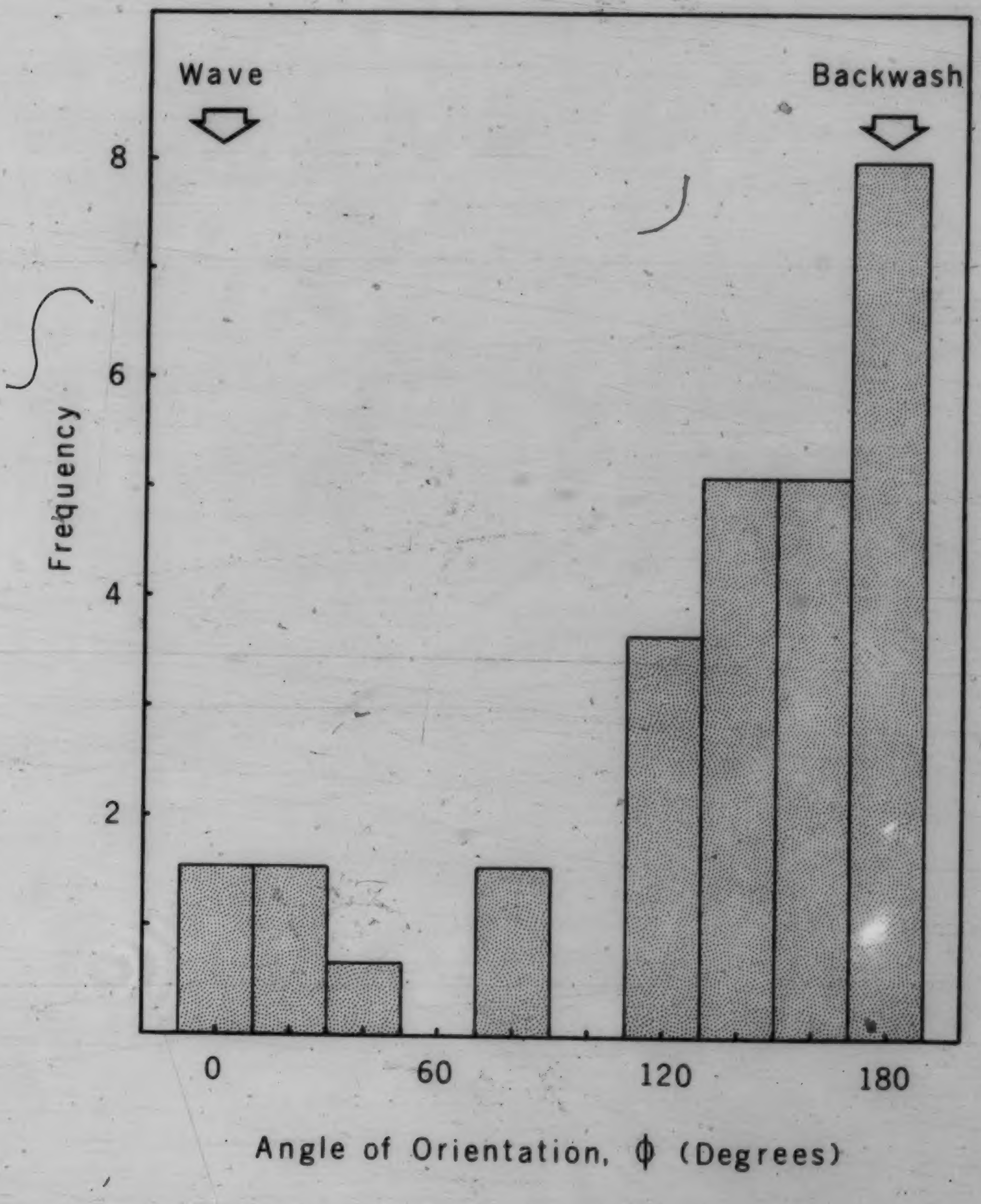

Wave
Backwash
Frequency
8
6
4
2
0
60
120
180
Angle of Orientation, ϕ (Degrees)

Figure 7. *Pollicipes polymerus*. Percent of clusters exhibiting mixed orientation as a function of cluster size (small, i.e., up to 35 animals; medium; or large, i.e., more than 150 animals) and substrate angle.

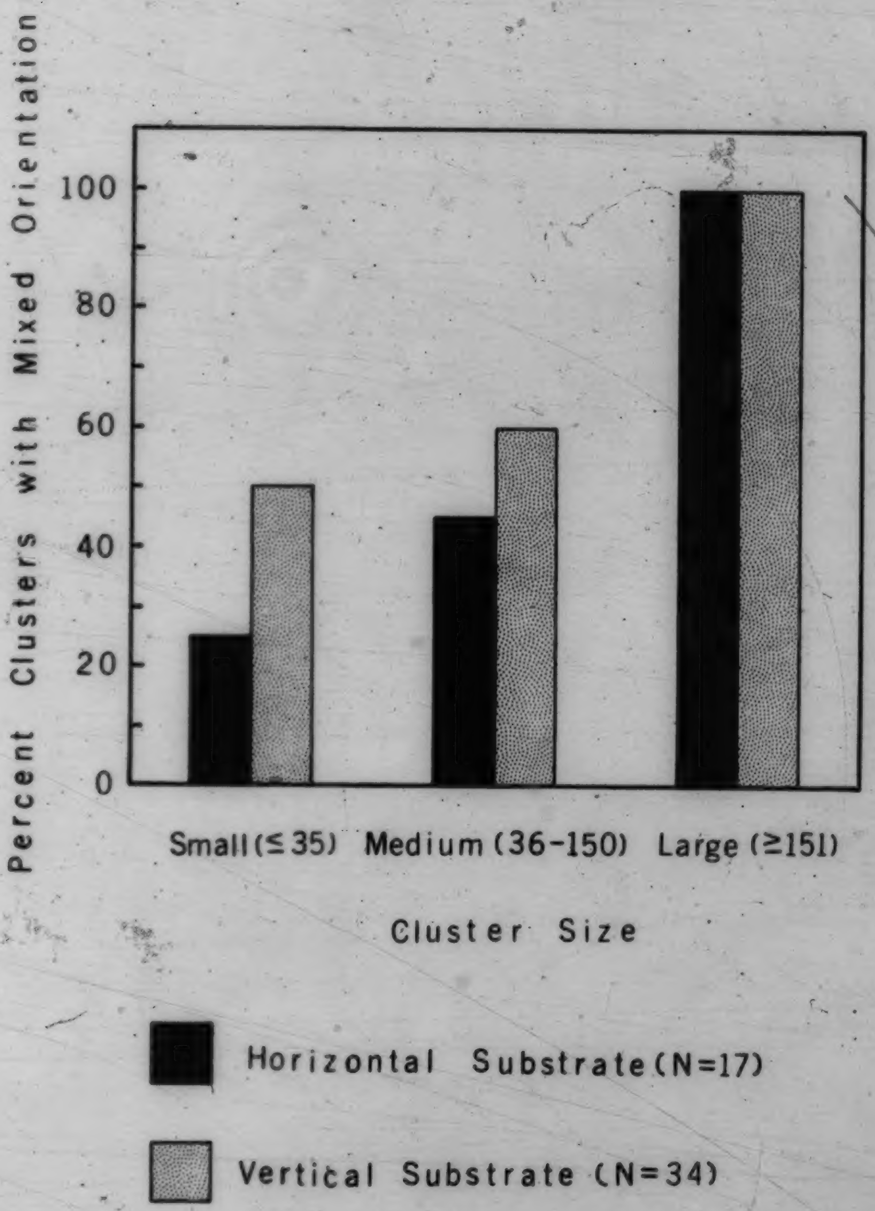
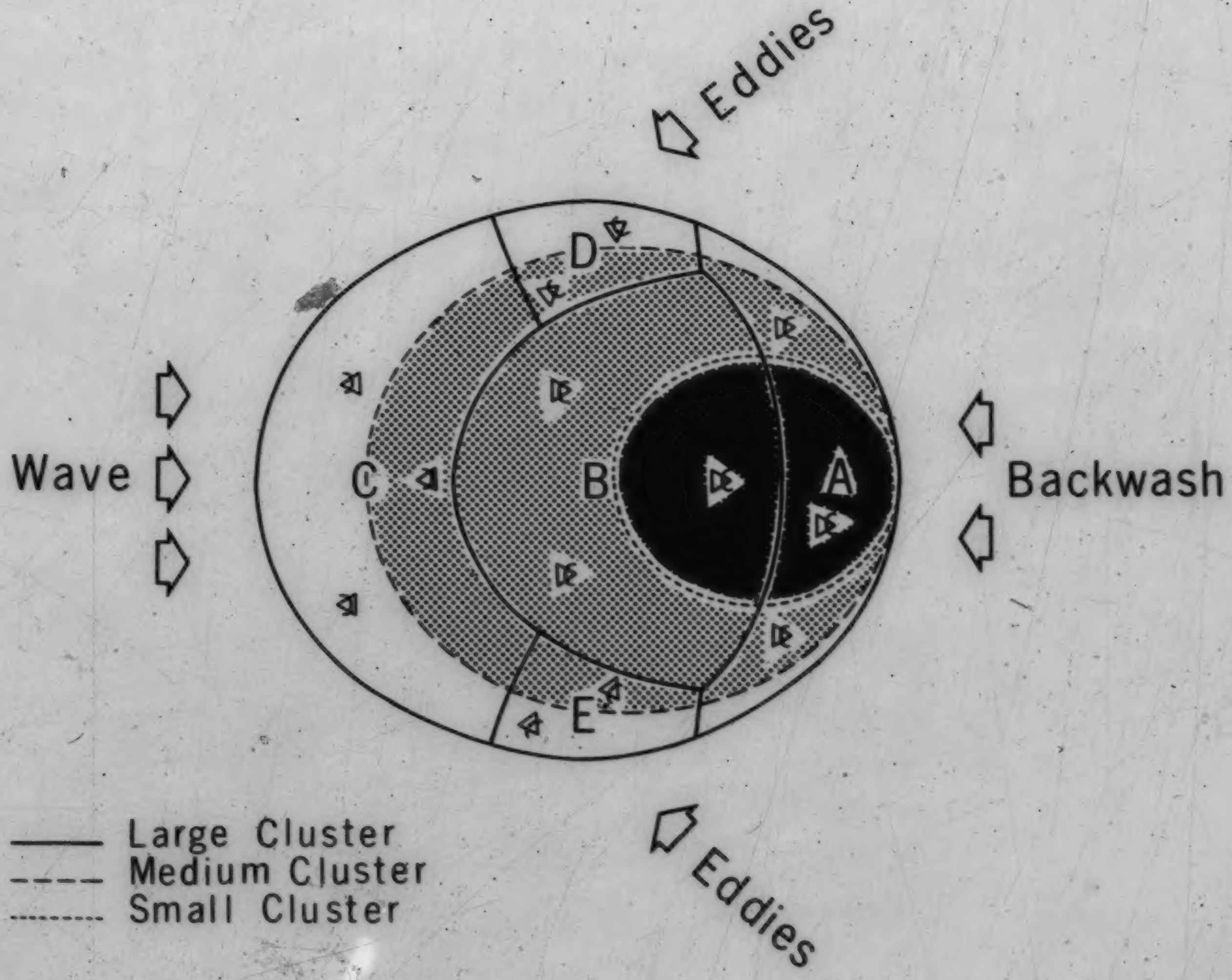

Percent Clusters with Mixed Orientation
0
20
40
60
80
100
Small (≤35)
Medium (36-150)
Large (≥151)
Cluster Size
■ Horizontal Substrate (N=17)
▨ Vertical Substrate (N=34)

Figure 8. *Pollicipes polymerus*. Model predicting orientation of the cirral net as a function of cluster size. The triangles with half circles superimposed represent barnacle capitula with cirral nets open. See text for explanation.


Eddies
Wave
A
B
C
D
E
Backwash
Eddies
Large Cluster
Medium Cluster
Small Cluster

Strength of orientation for the entire cluster is 0.31 (D and E were not calculated separately).

Discussion

Adult *P. polymerus* depend upon exogenous mechanical stimulation, such as waves or moving prey, for feeding (Howard, 1959; Barnes and Reese, 1959). Juveniles respond to these stimuli as well, but also beat their cirri without any apparent external stimulus, as do operculate barnacles.

Gwilliam and Bradbury (1971) suggested that the rhythmic burst of efferent nervous activity they recorded from all major trunks of the isolated central nervous system of *Balanus cariosus* are correlated with recurrent cirral beating. Donaldson and Prior (1972) recorded a regular pattern of spontaneous spiking activity at 12 to 15 second (sec) intervals while recording from the proximal end of the severed Cirral Nerve 1 of an adult *P. polymerus*. Nervous activity in juveniles was not recorded. It is possible that the spontaneous impulses in adults represent a remnant of the cirral beating pattern of juveniles, as this adult nervous activity approximates the rate of beat in 1 to 2 mm RC juveniles. This conclusion is in accord with the observations of Gwilliam and Bradbury (1971). It seems unlikely that this recorded rhythm represents the behavioral rhythm observed in adult *P. polymerus* by Barnes and Reese (1960), since these movements occur very slowly, every 50 to 100 sec.

The extension phase of the juvenile *P. polymerus* beat (79% of the total beat period) is longer than that in adult *B. balanus* (56%) (Crisp and Southward, 1961), and increases in length with growth of the animal, and finally ceases, after which only cirral extension is used.

Balanoids occasionally use cirral extension when exposed to strong currents (Southward and Crisp, 1958). In fact, some species of operculate barnacles (*B. balanoides* and *Elminius modestus*) vary their cirral activity with the amount of water current (Southward and Crisp, 1958). No "normal" cirral beating has been observed in adult *P. polymerus*, although they may occasionally "twitch" and "curl" their cirri in still water (Barnes and Reese, 1959). Since juveniles extend their cirri in fast currents and beat them in slow currents, environmental conditions appear to determine their mode of feeding behavior. It is of interest that the feeding mechanism of most lepadids is limited to cirral extension, and they are virtually restricted to habitats where there are fast water currents. In contrast, balanoids are capable of the full range of cirral activities and are able to occupy a greater diversity of habitats (Crisp and Southward, 1961).

Since beating behavior is observed only in the juveniles of *P. polymerus*, and they were found to have fed primarily on particulate food, the beating behavior seems to be a special microphagous adaptation. In fact, the food nets of smaller animals generally cover a greater area in relation to body size than do those of larger animals (Howard, 1959) and are, therefore, probably more efficient filters.

The preponderance of particulate material in the juvenile as compared to the adult gut is not due to inefficiency in capture of large food items, inasmuch as *Artemia* nauplii were easily consumed (see also Howard and Scott, 1959). The difference must then be due to method of capture and/or food availability.

Juveniles are almost always attached to adult peduncles (Lewis, 1975b), and thus, are situated below the mean adult cirral net level. In crowded areas where competition with macrophagous adults would be keen, juvenile beating behavior may be especially important in foraging. Southward (1955b) reported that feeding behavior may be related to habitat in some barnacles. The possibility of obtaining food by random contact would presumably be reduced until the young become large enough to compete successfully with adults. Although the adult extension macrofeeding behavior is generally more efficient than microfeeding (Crisp and Southward, 1961), juveniles may use cirral beating to maximize total feeding efficiency. This adaptation is probably necessary so that juveniles, sheltered from direct currents, may compete successfully with adults.

The proposed model for cirral orientation is consistent with the observation that most of the animals face the backwash, which provides a unidirectional current for any one barnacle cluster. The backwash usually has a long flow period, so that feeding time is greater than in the wave (Howard, 1959; Barnes and Reese, 1960). However, it is apparently advantageous for animals in large groups to exhibit mixed orientation. If all animals in a cluster turned toward the backwash, individuals in areas C, D and E would have to rely on food that escaped those animals anchored in areas A and B. Therefore, it would be to the advantage of some wave-side animals (C) to orient toward the waves, catching food particles as they are swept inshore and, not to compete with the rest of the population. Animals in area B consistently have the longest peduncles (Howard, 1959), so probably compete successfully with animals in area A. Animals in areas D and E may take advantage of eddy currents splashing across the cluster (edge effects).

In *P. polymerus* exposed to water currents in the laboratory, those nearest water jets respond the most by rotating toward the currents so as to face them directly, whereas animals shielded by the rest of the group turn only slightly (Barnes and Reese, 1960). Thus, in semi-exposed areas such as San Juan Island, it is not unusual to find that 21% of the members of large barnacle populations face waves directly. The model is consistent with these observations since areas A and B shield areas C, D and E from the backwash, allowing areas C, D and E to be more strongly influenced by other currents. As one would expect, in clusters with a small number of animals this effect is less noticeable.

It is possible that *P. polymerus* have the most freedom of movement when young, especially if in a less crowded situation. Most young *P. polymerus* would probably orient toward the dominant backwash current. As the cluster grows larger and tighter, there is less flexibility for full capitulum rotation. Thus, orientation of animals in area B may be relatively fixed, while the peripheral groups are more free to change orientation with changes in current direction.

Although other authors have noted the orientation of operculate barnacle cirri toward waves (Moore, 1933, 1935; Hiro, 1939; Crisp, 1953; Crisp and Stubbings, 1957), only 2 such results have been reported for pedunculates: *Conchoderma*, a whale barnacle, was oriented on a fish, *Diodon*, "to receive the full benefit of currents derived from . . . forward swimming" (Crozier, 1916); and *P. polymerus* was recorded to be similarly oriented in a situation where there was no backwash

(Senechal, 1969). The present study indicates that it is possible to describe orientation of *P. polymerus* by a model which is in agreement with accounts in the literature. According to the model, mixed orientation permits maximum feeding efficiency for animals in each component of the cluster, at least in the semi-exposed areas studied. It is possible that waves hitting extended cirri in exposed, outer coast situations apply more force than most extended animals can bear. Thus, fewer animals face the waves.

GROWTH AND REPRODUCTIVE BIOLOGY

Introduction

Since some groundwork has been laid in the study of the reproductive biology of *Pollicipes polymerus* in the central and southern portions of the species' distribution (Hilgard, 1960; Straughan, 1971; Hand *et al.*, 1973), it is felt that an analysis of various aspects of reproduction at the northern end of the animals' range for the purpose of comparison is worthwhile. Moreover, some of the basic questions with regard to the effect of environmental factors on reproduction still remain unanswered. In addition, 1 study shows that *P. polymerus* grows extremely slowly in southern California (Barnes and Reese, 1960). Data is given in this chapter which may be compared with what is known. This study was carried out at the Friday Harbor Laboratories, Washington, from July, 1971 to August, 1973, and is designed to determine: 1) size of the animal at reproductive maturity; 2) mode of fertilization; 3) possible exogenous stimulus to copulation; 4) possible variation of embryo brooding cycles and fecundity through different latitudes; 5) possible variations of brooding cycles and fecundity among adjacent populations; and 6) possible environmental effects on brooding and fecundity. All data were obtained by sampling field populations, since a method for keeping *P. polymerus* in a "natural state" in the laboratory for long periods has not yet been developed, and breeding in the laboratory has not yet been successfully accomplished.

P. polymerus is an ovoviviparous hermaphrodite. The finding of embryo masses brooded in the mantle cavity was taken as an indication that breeding activity had preceded. Since copulation of this barnacle has never been witnessed, all further reference to reproductive activity will be made to the "brooding" of embryos.

Materials and Methods

Growth

The growth of invertebrates is often studied by following changes in a linear dimension and subsequently converting the measurements into estimates of mass. In this study rostral-carinal length was chosen over tergal or scutal-tergal length because the data were less scattered.

Isolated individuals are rare; their growth rate may not reflect the normal pattern. Thus, 9 distinct clusters containing a total of 84 animals were marked with red enamel paint in July and August, 1971. Rostral-carinal length was subsequently measured monthly for a year with a pair of Starrett dividers, estimating to the nearest 0.1 mm. A final measurement was made after 12 more months had elapsed.

The relationship between RC length and dry weight was calculated for animals of various sizes at both study localities. The data were transformed to logarithms and a regression line was fitted using the orthogonal least squares method (Daborn, 1974). Calculation of the coefficient of determination (r^2) showed that about 90% of the variation in dry weight may be explained by length.

Reproductive Biology

Isolated adults were collected during the reproductive season (July and August, 1971) and were dissected to determine whether they brooded embryos or not in relation to distance (measured between peduncle bases) from the nearest mature individual. Various sizes of adults were collected to determine the size at which reproductive maturity occurred.

Copulation. It was assumed that cross-fertilization occurs at least part of the time. Experiments were conducted to determine if copulation could be stimulated in the laboratory and in the field. Clusters of adults of sexually mature size were removed to the laboratory. The animals were left out of water for varying periods of time and then were reimmersed and observed for copulatory activity (Walley *et al.*, 1971; J. Rees, Personal communication). 0.1% ascorbic acid and saturated solutions (33%) of ascorbic acid in sea water were poured on healthy adults in the laboratory tanks and in tidal pools to see if copulation was stimulated as in some acorn barnacles (Collier *et al.*, 1956; Barnes and Finlayson, 1962).

Brooding cycle. Four populations were sampled for the percent brooding at approximately monthly intervals over 26 months: Edward's Reef at high (+6.0) and low (+3.0) intertidal levels, and Eagle Point at high (+5.0) and low (+1.0) intertidal levels. Only animals of reproductively mature size (RC length greater than or equal to 17 mm at Eagle Point and 14 mm at Edward's Reef) in dense clusters were collected and fixed in 70% ethanol. Using animals collected from dense clusters eliminates bias in case self-fertilization does not occur.

Comparisons of percent brooding were first made between the 4 populations by month and total of all months recorded using the

2 X 2 Chi Square Test of Independence (with G-statistic when there were small samples). However, since the high and low intertidal heights do not coincide exactly at the 2 localities monitored, it is probably not valid to compare intertidal height between areas, only within an area. For this reason, the F-Test for analysis of variance (Steele and Torrie, 1960) was subsequently used to test the effect of 1) time of the year, 2) population locality, 3) intertidal height within locality, and 4) interaction of month with locality upon the percent of barnacles brooding. Two assumptions were made before performing the F-Test: 1) normal distribution of data and 2) homogeneity of variance. It is not known whether or not the data in question has a normal distribution, but it has been shown that if sample sizes are equal or nearly equal, the test is still robust (Cochran, 1947). Sample sizes used in all F-Tests were equal. The Hartley's F-maximum test determined that the data also fulfilled the homogeneity of variance requirement. Since a significant monthly difference was detected, the Newman-Keuls pairwise comparison test was performed to determine differences between individual months of the reproductive season. After determining that highly significant differences in reproductive activity occurred between animals at the 2 localities, but not between animals at different intertidal heights within either locality, the 2 intertidal groups were combined at each locality and subsequently compared using the 2 X 2 Chi Square Test of Independence. The exact months during which significant differences occurred were then determined.

Air temperature was calculated by taking the minimum and maximum for 5 consecutive days from daily temperatures recorded in the Friday

Harbor Journal. Subtidal (15 to 60 feet) sea water temperature was recorded by C. Nyblade at monthly intervals at Edward's Reef or Mitchell Point. Surface water temperature is taken from the following sources: Scripps Institute of Oceanography/U.S. Naval Hydrographic Office for 1943, calculated from 5°F isotherms of oceanic and coastal water in Puget Sound; S. Woodin for 1969-70, University of Washington, Doctoral Thesis, monthly means from a cove on San Juan Island; Thompson and Phifer for 1931-35, monthly means at Cantilever Pier, Friday Harbor Laboratories; and my own recording for 1971-73, average of Eagle Point and Edward's Reef monthly recordings at low tide. Day length was calculated from the sunrise and sunset tables for Port Townsend, Washington. Total daily immersion times were calculated by fitting a sinusoidal curve to the 4 daily tidal high and low points using a Fortran program (Appendix I).

Fecundity. Fecundity is a reflection of fertility or productivity which may be measured as the number of embryos or broods produced per adult per reproductive period. The mean monthly dry weight of brooded embryos was compared using the F-Test as above. In addition, correlation coefficients for embryo mass dry weight to adult linear size and embryo mass dry weight to embryo stage were calculated to determine the extent of the relationships. Further calculation provided estimates of fecundity.

RESULTS

Growth

Rostral-carinal length is related to dry weight in *P. polymerus* by the equation: $Weight\ (g) = 2 \times 10^{-4}\ Length^{3}\ (mm)$ (Fig. 9). Since $length^{3}$ is approximately proportional to the animals' volume, it appears that weight and volume are directly proportional, and further, that growth is allometric (Winberg, 1971).

Mean RC measurements followed over a 2 year period show that an animal may grow as much as 11 mm in the first year (Fig. 10). The mean size in 1 population at Eagle Point increased from 4 to 15 mm in a single year (Fig. 11). Since the cypris is about 0.5 mm long (Lewis, 1975b), it is conceivable that settlement had occurred for these small animals in May or June of the same year (1971). Above about 13 mm, growth rate appears to level off to 1 to 2 mm per year. The range of times to sexual maturity (14 or 17 mm RC) was estimated. Since it is not known whether it takes 2 months or 1 year after settlement to reach 4 mm RC, a range of 3 to 6 years between cyprid settlement and attainment of sexual maturity is possible. However, when *P. polymerus* was completely removed from a plot on the West Coast of Vancouver Island, 17 mm *P. polymerus* were found 12 months later (Marine Biology class project, University of Alberta). Thus, it probably takes 1 year to maturity at that location. Another 4 to 8 years may elapse before 25 mm RC (average adult size) is reached at San Juan Island. It is not known what the total life expectancy is, since animals in the largest size range were not followed for more than 2 years. It is presumed from the data given here that *P. polymerus* may reproduce for at least 10 years and possibly for 15 years.

Figure 9. *Pollicipes polymerus.* Relationship between rostral-carinal length and dry weight. The least squares regression line is given.

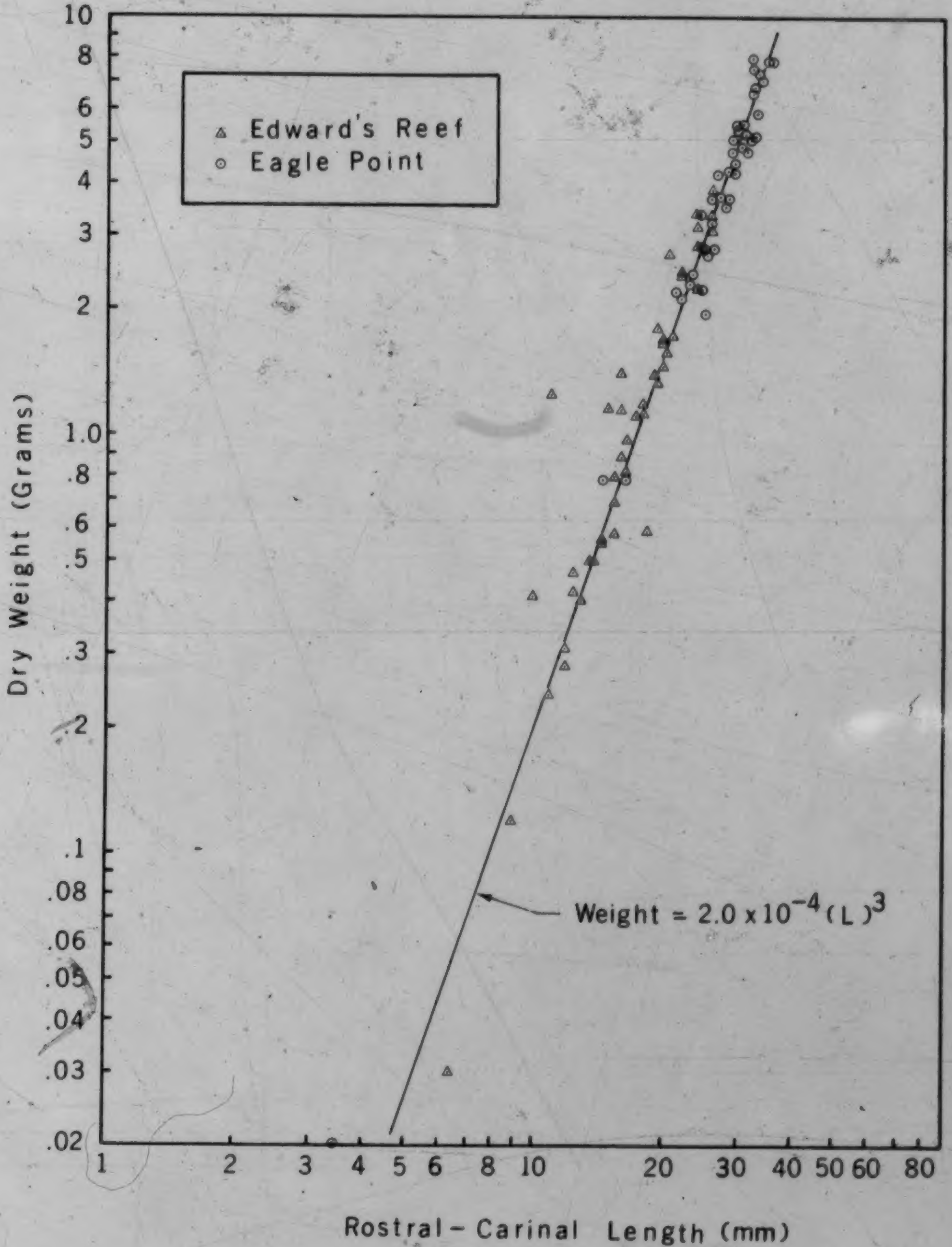

Edward's Reef
Eagle Point
Dry Weight (Grams)
10
8
6
5
4
3
2
1.0
.8
.6
.5
.3
.2
.1
.08
.06
.05
.04
.03
.02
Weight = $2.0 \times 10^{-4} (L)^3$
1
2
3
4
5
6
8
10
20
30
40
50
60
80
Rostral - Carinal Length (mm)

Figure 10. *Pollicipes polymerus.* Growth *in situ* at Edward's Reef over 26 months. Size of marked juvenile and adult animals at high, middle and low intertidal levels was periodically recorded and the mean plotted for each group. Sample sizes are given at the end of each curve.

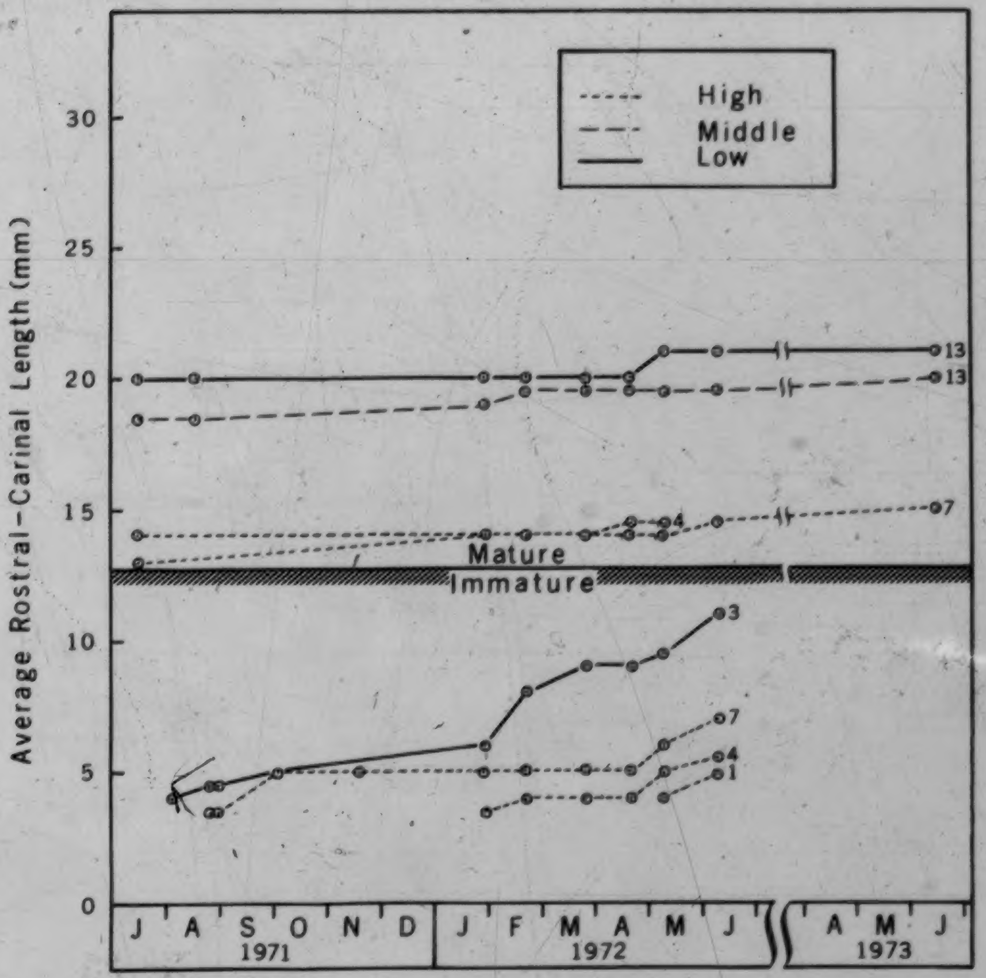
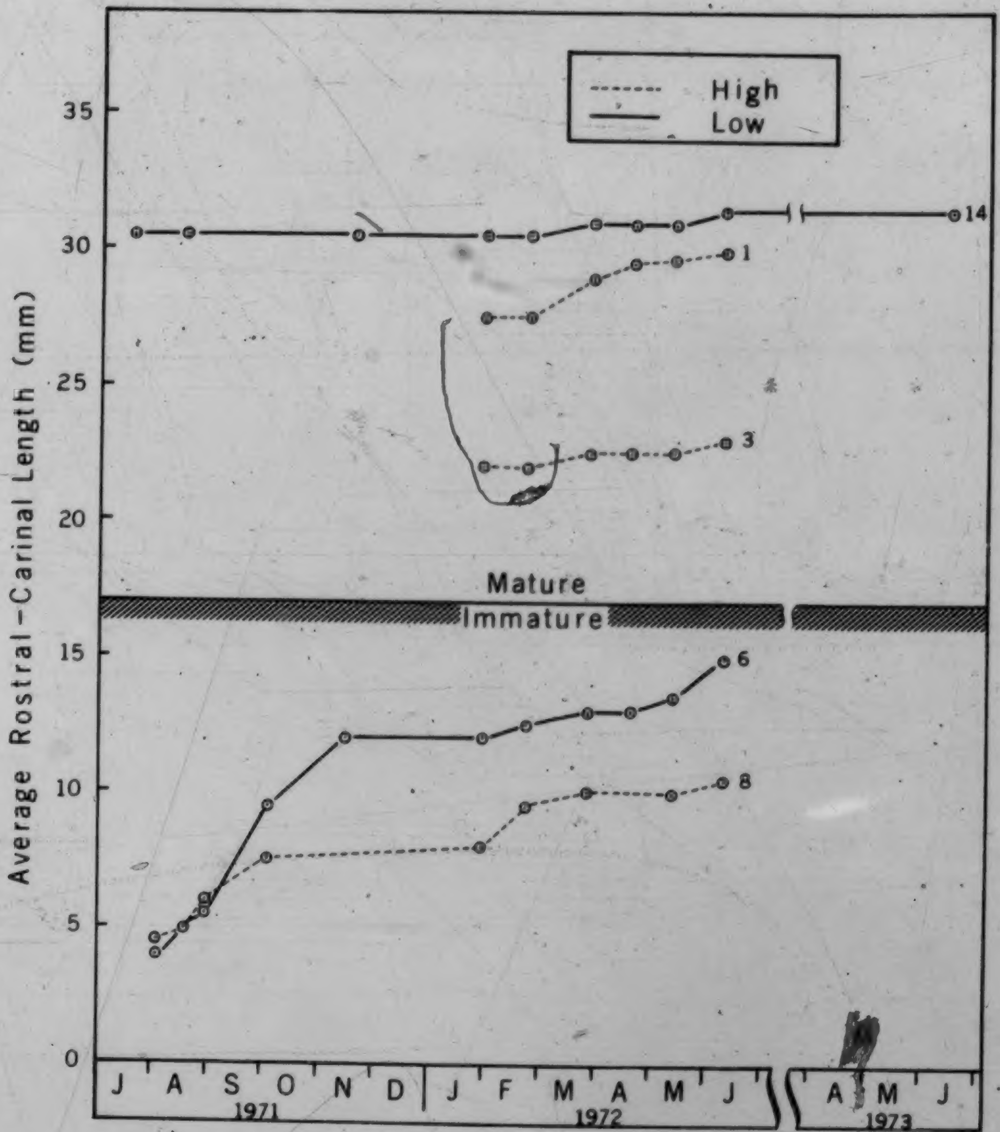

High
Middle
Low
Average Rostral-Carinal Length (mm)
30
25
20
15
10
5
0
13
13
7
4
Mature
Immature
3
7
4
1
J
A
S
O
N
D
1971
J
F
M
A
M
J
1972
A
M
J
1973

Figure 11. *Pollicipes polymerus*. Growth *in situ* at Eagle Point over 26 months. Size of juvenile and adult animals at 2 intertidal levels was recorded and the mean plotted for each group. Sample sizes are given at the end of each curve.



High
Low
Average Rostral-Carinal Length (mm)
35
30
25
20
15
10
5
0
14
1
3
6
8
Mature
Immature
J
A
S
O
N
D
J
F
M
A
M
J
A
M
J
1971
1972
1973

P. polymerus grows fastest in winter and fall (Figs. 10 and 11), attaining an average of 13 mm (9 to 15 mm) during the first year in all populations measured. The lower intertidal juvenile individuals at both sites reached a larger size than the corresponding high intertidal populations.

Reproductive Biology

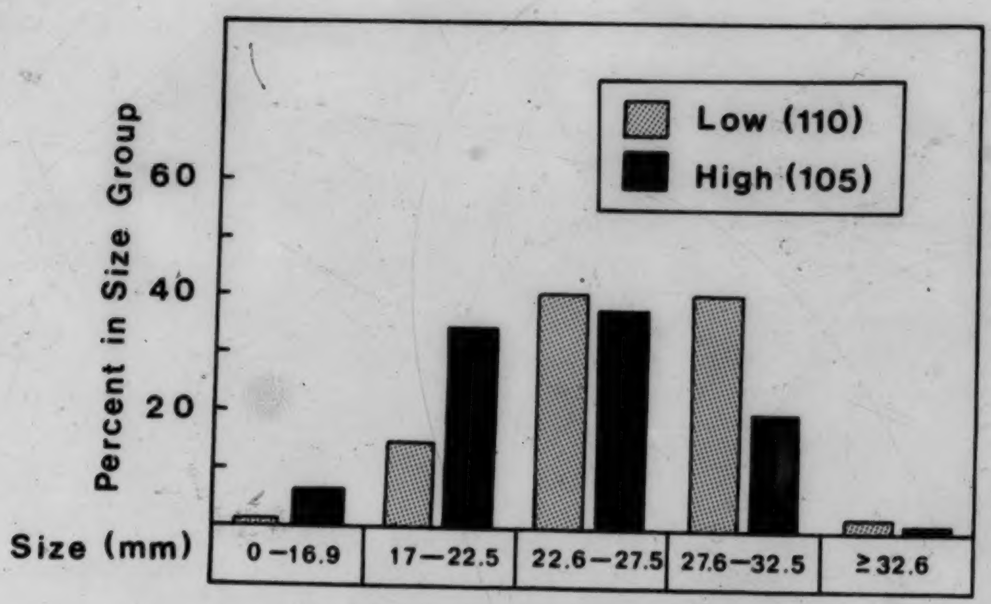
Size at sexual maturity. Animals sampled for occurrence of ovigerous lamellae (egg masses) were arbitrarily assigned to groups by size. These data indicate that animals between 17 and 32.5 mm RC constitute the major proportion of the breeding population at Eagle Point and those between about 14 and 27.5 mm RC constitute the breeding population at Edward's Reef (Fig. 12). Thus, only animals within these size ranges were included in the study of reproduction and fecundity.

Required distance for brooding. The results of the field study designed to determine if isolated animals carried brooding embryos during the reproductive season indicate that over 60% of closely grouped (up to 5 cm apart) animals contained ovigerous lamellae. Animals separated by more than 11 cm did not contain embryos and between 5 and 11 cm brooding activity was inversely proportional to distance (Fig. 13). Extrapolating from these results, it seems unlikely that self-fertilization is occurring in isolated individuals in the study sites.

Brooding cycle. The first brooded embryos of *P. polymerus* are seen in April at San Juan Island. There is a general buildup of brooding activity to the peak of the season in July, after which brooding decreases until it ceases in November. No brooding occurs from November to March (Fig. 14). The proportion of animals in all populations brooding embryos

Figure 12. *Pollicipes polymerus*. Size frequency distribution of the brooding animals from June through August in the 4 populations monitored. Sample sizes are in parentheses. The top graph depicts animals at Eagle Point; the bottom graph depicts animals at Edward's Reef.

7


Low (110)
High (105)
Percent in Size Group
60
40
20
Size (mm)
0-16.9
17-22.5
22.6-27.5
27.6-32.5
≥32.6

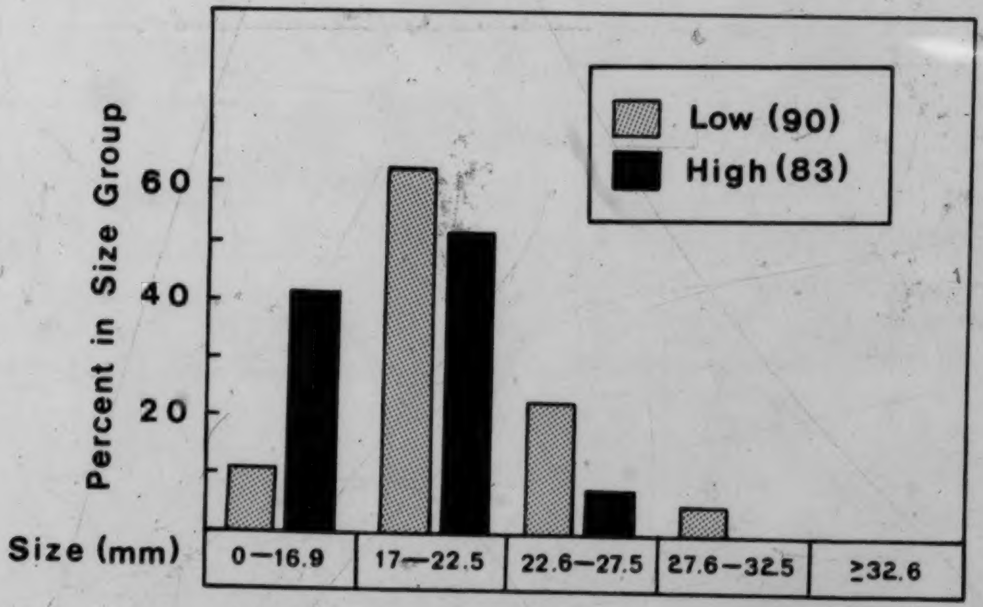

Low (90)
High (83)
Percent in Size Group
60
40
20
Size (mm)
0-16.9
17-22.5
22.6-27.5
27.6-32.5
≥32.6

Figure 13. *Pollicipes polymerus*. Percentage of mature adults containing ovigerous lamellae when separated from other adults by given distancesduring the normal breeding period (July and August) in 1971. Numbers at each plotted point indicate sample sizes.

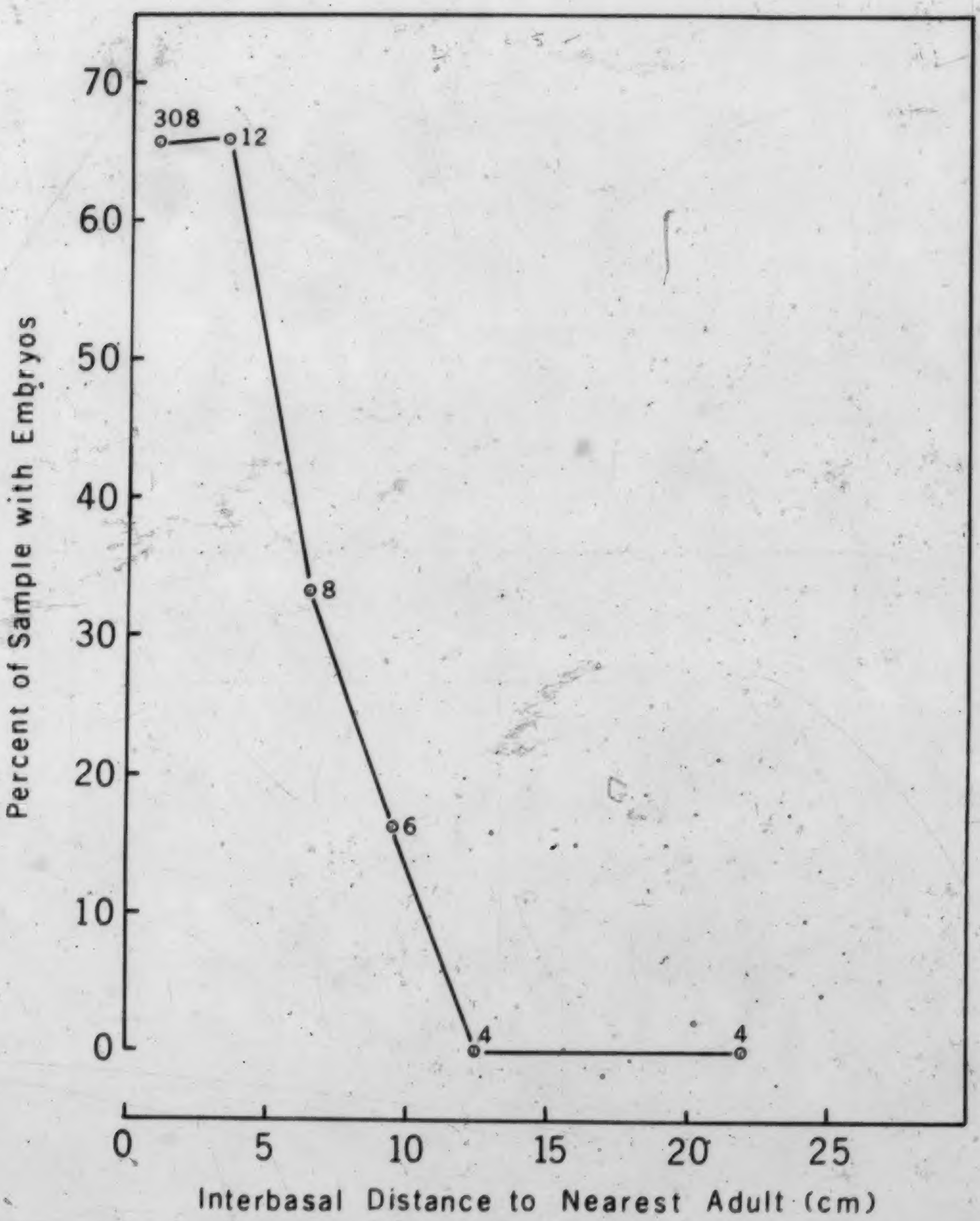
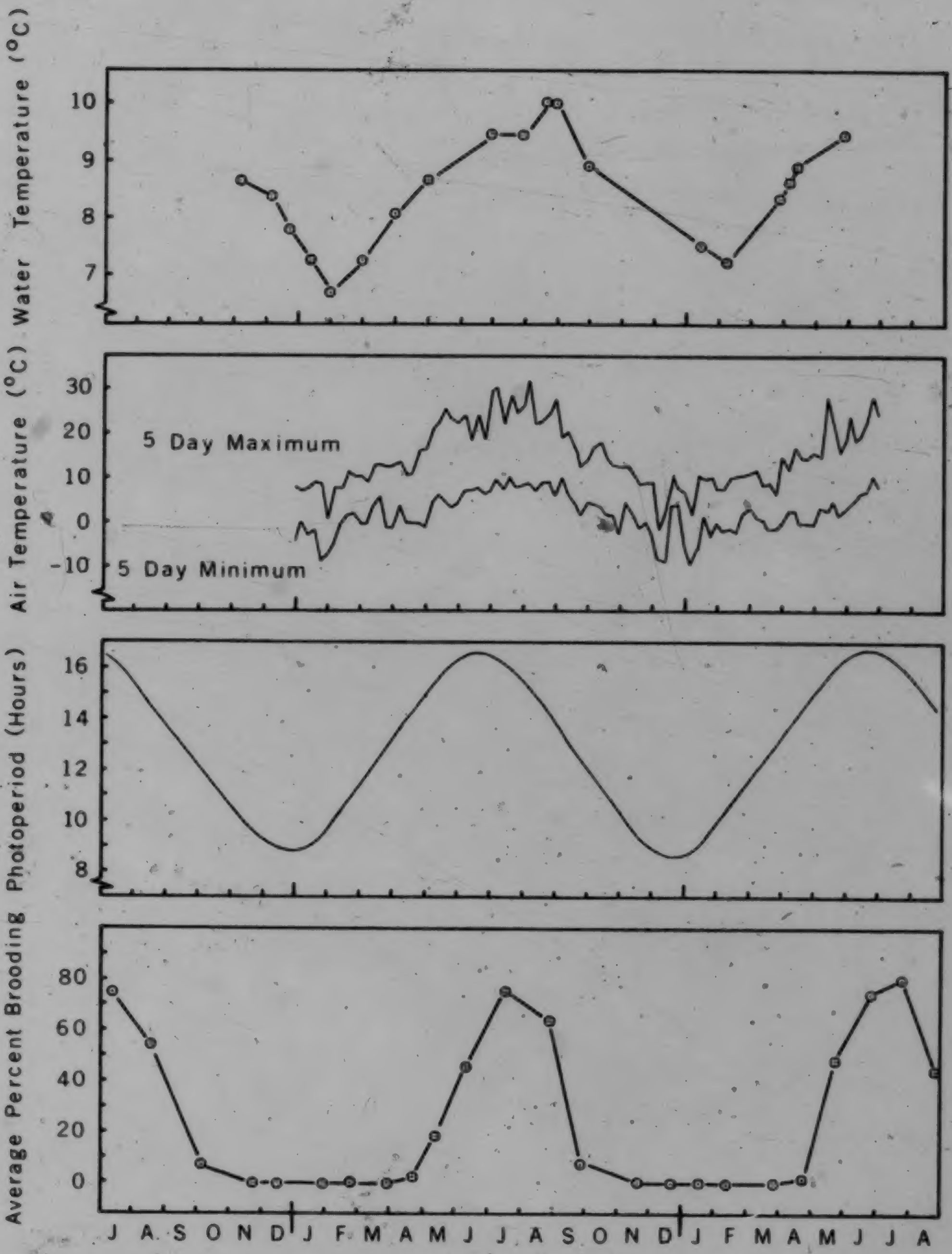

Percent of Sample with Embryos
70
60
50
40
30
20
10
0
308
12
8
6
4
4
0
5
10
15
20
25
Interbasal Distance to Nearest Adult (cm)

Figure 14. *Pollicipes polymerus*. Reproductive cycle over 26 months at San Juan Island, Washington, and compared to: photoperiod, air temperature, and sea water temperature (subtidal 15 to 60 feet). Percent brooding is the average of the 4 populations sampled at San Juan Island.


Water Temperature (°C)
10
9
8
7
Air Temperature (°C)
30
20
10
0
-10
5 Day Maximum
5 Day Minimum
Photoperiod (Hours)
16
14
12
10
8
Average Percent Brooding
80
60
40
20
0
J A S O N D J F M A M J J A S O N D J F M A M J J A

for a 26 month period at San Juan Island closely follows the 4 environmental parameters graphed: day length, air and surface as well as sub-surface sea water temperature (Figs. 14 and 15). Brooding begins when the sea water temperature is about 8° to 9°C and rising, and stops at 9° to 10°C when the temperature is declining.

Most populations contain individuals brooding all embryonic stages during July and August. The last brood of the season is during October at Eagle Point (Fig. 16) and during September at Edward's Reef (Fig. 17). Early and middle stage embryos dominate in April, May and June at Eagle Point, while they are not seen until May at Edward's Reef.

Eagle Point populations probably begin new broods through September, while only late stages are observed in September at Edward's Reef. Thus, the reproductive season lasts 1 to 2 months longer at Eagle Point than at Edward's Reef. Differences in brooding patterns between high and low populations within each locality may reflect sampling error only, since there are no statistically significant differences in percent brooding between these groups.

Copulation. Freshly collected animals were immersed after approximately 24, 48 or 72 h of exposure to air. They were placed together in tanks of running sea water for maximum opportunity to copulate; however, no such activity was observed. Fifty-four animals were immersed after exposure to air for 24 h and observed at least every 15 min for 2 h and 45 min in May, 1972, at San Juan Island. Of the 17 animals subsequently dissected (31.5%), none harbored sperm masses. After 48 h of exposure to air, 2 groups were observed during 4 h of immersion. Thirty animals (60%) were then dissected in 1 group and 22 (39.3%) in a second group; none held recently fertilized eggs or

Figure 15. *Pollicipes polymerus*. Surface sea water temperature in Puget Sound from 4 sources.

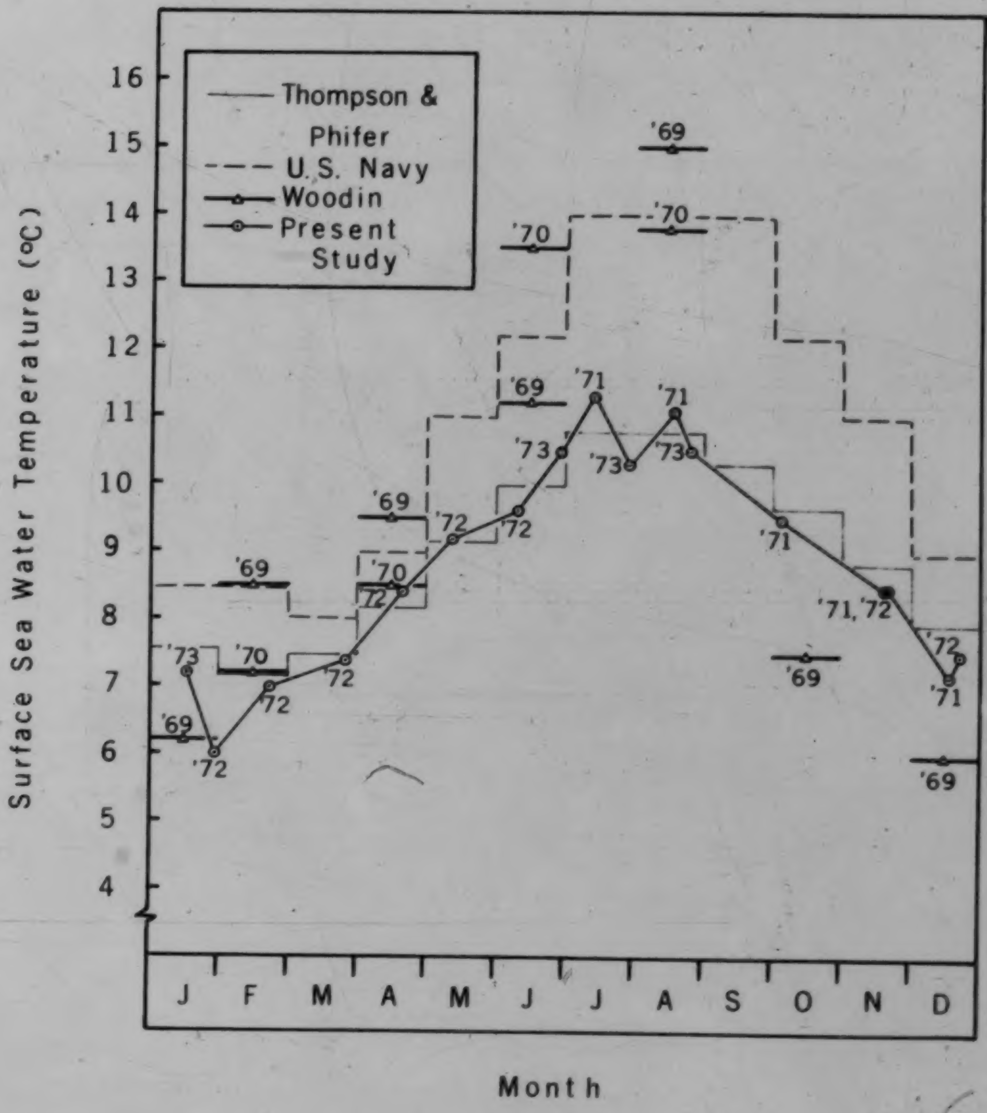

Thompson & Phifer
U.S. Navy
Woodin
Present Study
Surface Sea Water Temperature (°C)
Month
16
15
14
13
12
11
10
9
8
7
6
5
4
J
F
M
A
M
J
J
A
S
O
N
D
'69
'70
'71
'72
'73
'71, '72

Figure 16. *Pollicipes polymerus.* The embryonic stages found in the ovigerous lamellae from the adults at Eagle Point over 26 months, showing percent of the total sample of adults. Early embryos encompass zygotes to the formation of naupliar segments, late embryos have developed the naupliar eye, and middle embryos are between segmentation and eye formation.

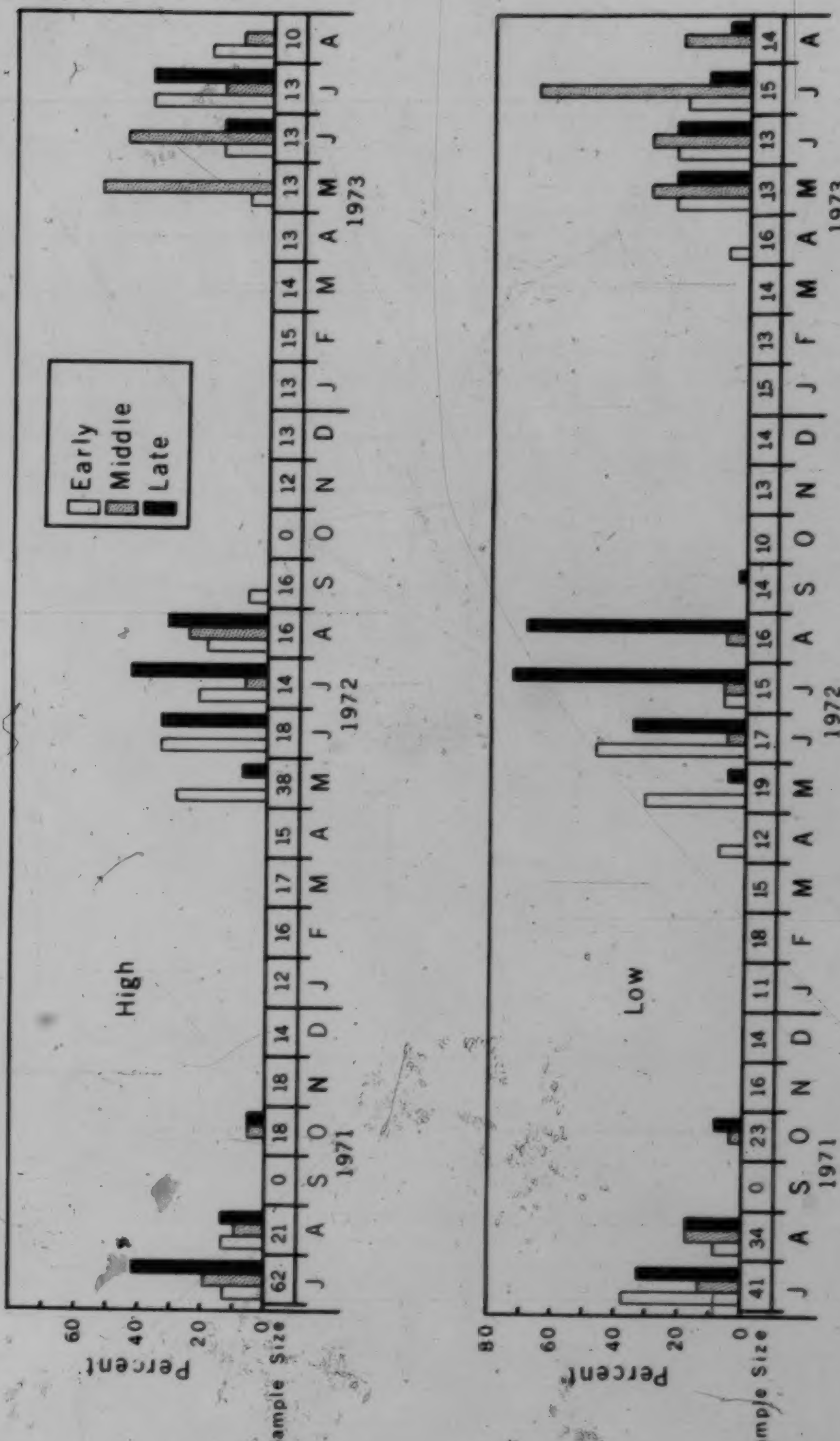
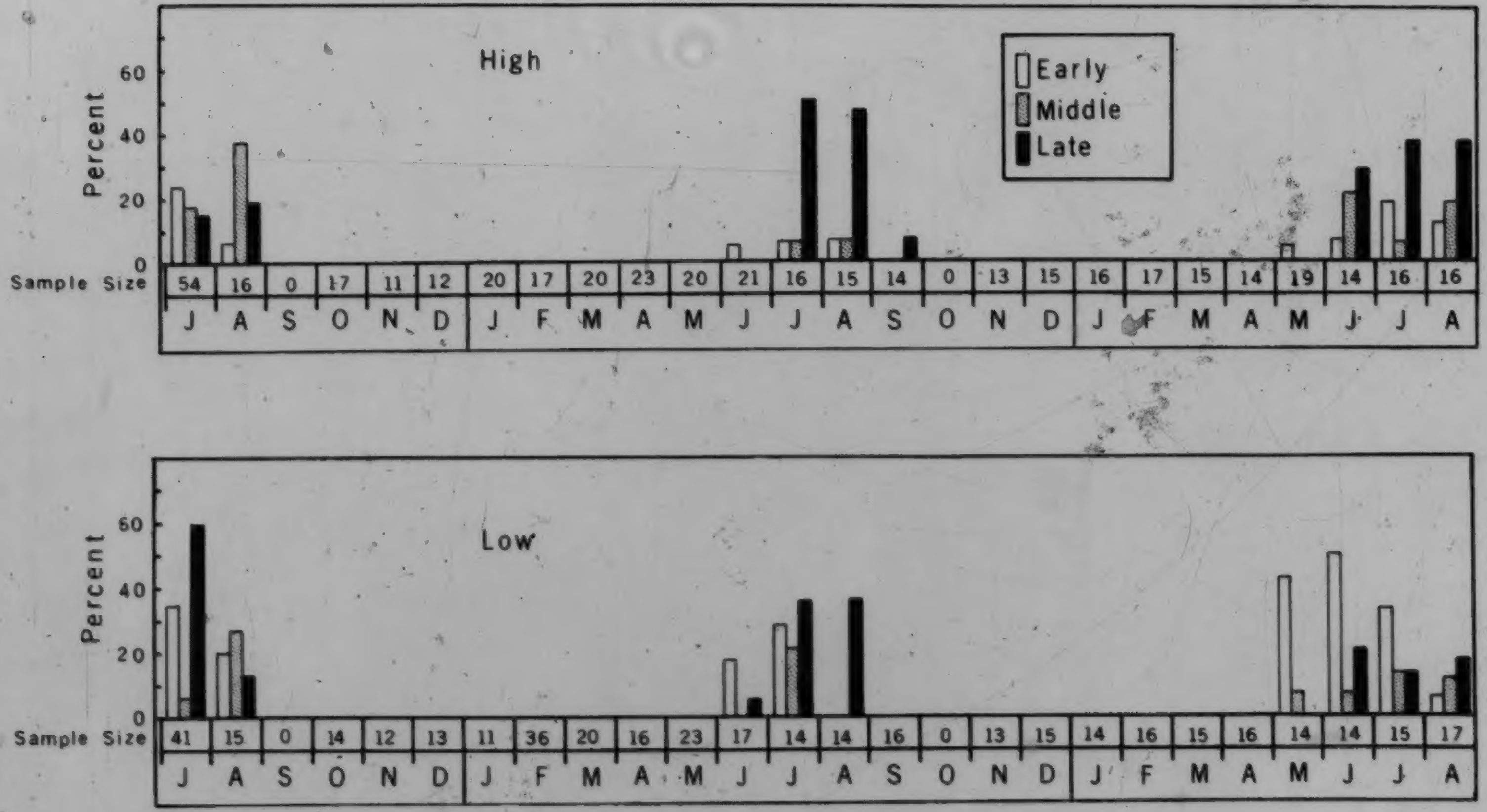

Early
Middle
Late
High
Low
Percent
Sample Size
0
20
40
60
80
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
1971
1972
1973
62 21 0 18 18 14 12 16 17 15 38 18 14 16 16 0 12 13 13 15 14 13 13 13 13 10
41 34 0 23 16 14 11 18 15 12 19 17 15 16 14 10 13 14 15 13 14 16 13 13 15 14

Figure 17. *Pollicipes polymerus*. The embryonic stages found in the ovigerous lamellae from the adults at Edward's Reef over 26 months, showing percent of the total sample of adults.


High
Low
Percent
0
20
40
60
Sample Size
Early
Middle
Late
54 16 0 17 11 12 20 17 20 23 20 21 16 15 14 0 13 15 16 17 15 14 19 14 16 16
41 15 0 14 12 13 11 36 20 16 23 17 14 14 16 0 13 15 14 16 15 16 14 14 15 17
J A S O N D J F M A M J J A S O N D J F M A M J J A

implanted sperm. After 72 h of exposure to air, 47 animals were observed for copulatory activity for 3 h. Twenty-eight animals (59.6%) were dissected; none contained sperm masses. A similar set of observations made at Bodega Bay Marine Station in June, 1972, supported these results.

Furthermore, animals were freshly collected at Windmill Beach, California, and placed in large finger bowls to which 0.1% ascorbic acid in sea water was added. In 2 animals sperm were observed projecting from the capitulum 10 min after addition of the vitamin. One animal subsequently extended its cirri part-way 4 times, closing them again after each time. At no time were exploration, probing and insemination (Wong, 1967) observed. Both animals were dissected and contained freshly ovulated, unfertilized eggs, and sperm implanted in the mantle cavity. The sperm were found to be mature, although inactive. The same concentration of ascorbic acid produced no effect on animals immersed in sea water for 48 h. Saturated solutions of ascorbic acid introduced to animals in the laboratory and in a partially full tidal pool into which waves occasionally washed produced no copulatory activity.

A higher proportion of freshly inseminated animals was found on May 24, 1972, at Eagle Point than in previous experience. The tide was in a receding series and was lowest at +0.4 feet on this day. On the assumption that tidal level might play some part in triggering copulation, other collections were made during low tide at Eagle Point and at Doran and Windmill beaches near Bodega Bay, California, in June, 1972. Two percent or less of a given population is freshly fertilized during a receding tidal series on the first low tide of about 0.0 feet in these areas (Table 3). When total daily immersion times were compared with the period of first breeding, no correlation could be detected (Figs. 62 and 63, Appendix I).

Variation in the brooding cycle. The total proportion of adults brooding embryos over 26 months is greatest in the Eagle Point low group and decreases (descending order) in the Eagle Point high; Edward's Reef low; and Edward's Reef high groups (Fig. 18). This pattern is the same as observed for adult size and juvenile growth rate. Individuals at Eagle Point begin brooding in April, while populations at Edward's Reef do not begin until May or June (Fig. 18). Both Eagle Point groups continue brooding into October, while those at Edward's Reef finish in September. In addition to the San Juan Island populations, different proportions of brooding individuals were found in 2 habitats in June, 1972, near Bodega Bay, California (Table 3).

The F-Test shows that the brooding rates differ significantly in animals at the 2 localities ($p < .001$), but not in animals at different intertidal heights in the same locality. A significant difference in percent brooding is also detected when months are compared ($p < .001$). When plotted over time, percent brooding describes a bell-shaped curve with a peak in July. Observations at monthly intervals always showed that there is a significant change (Newman-Keuls Test, $p < 0.05$). The 2 X 2 Chi Square Test shows that percent brooding differs significantly between animals at Edward's Reef compared to animals at Eagle Point (Table 4) at the beginning or peak of the reproductive cycle.

There is a difference in the numbers of species associated with *Pollicipes* at the 2 study sites (Table 5) which may be related to the 2 physical differences between Eagle Point and Edward's Reef: 1) wave action

Table 3. *Reproductive condition of* Pollicipes polymerus *from 4 sites. Number of specimens examined is in parentheses.*

Date	Area	Tidal level at time of collection		Without embryos	Ovulating	With developing embryos	With sperm freshly deposited; no eggs in mantle cavity
May 24, 1972	Eagle Point	+0.4	(10)	10	10	0	0
June 7, 1972	Eagle Point	+0.1	(220)	119	3	90	8
June 13, 1972	Doran Beach, high	-0.8	(94)	89	0	5	0
June 13, 1972	Doran Beach, low	-0.8	(48)	37	1	8	2
June 17, 1972	Windmill Beach	+0.4	(436)	195	10	201	30

Figure 18. *Pollicipes polymerus*. Percent of individuals brooding in 4 populations on San Juan Island over 26 months.

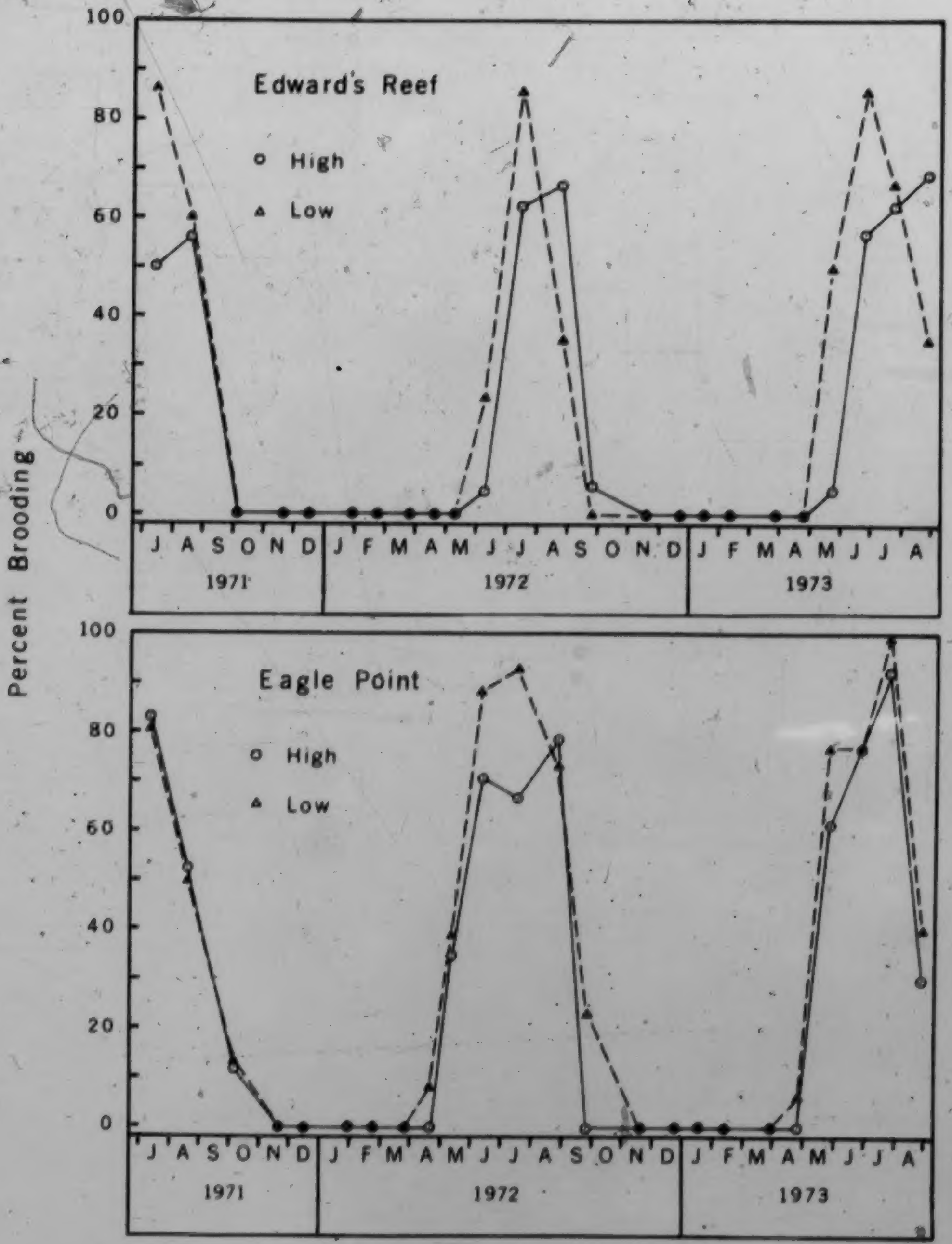

Percent Brooding
Edward's Reef
o High
▲ Low
100
80
60
40
20
0
J A S O N D J F M A M J J A S O N D J F M A M J J A
1971
1972
1973
Eagle Point
o High
▲ Low
100
80
60
40
20
0
J A S O N D J F M A M J J A S O N D J F M A M J J A
1971
1972
1973

Table 4. *Comparison of percent gooseneck barnacles brooding embryos at 2 localities over a 26 month period using a* χ^2*-test. NS, not significant;* $*P < 0.05$; $**P < 0.001$.

Date	Edward's Reef No. brooding/No. not brooding	Eagle Point No. brooding/No. not brooding	Level of significance
July, 1971	54/28	56/12	*
August	18/13	27/26	NS
October	0/35	5/34	NS
November	0/22	0/27	
December	0/33	0/25	
January, 1972	0/31	0/23	
February	0/53	0/24	
March	0/36	0/28	
April	0/39	1/26	NS
May	0/43	14/24	**
June	5/33	27/7	**
July	22/8	19/4	NS
August	15/14	22/7	NS
September	1/32	4/25	NS
November	0/26	0/24	
December	0/30	0/27	
January, 1973	0/30	0/25	
February	0/33	0/25	
March	0/30	0/26	
April	0/30	1/26	NS
May	8/25	18/8	**
June	20/8	20/6	NS
July	20/11	27/1	*
August	17/16	7/17	NS

Table 5. *Species found within clusters of* Pollicipes polymerus *at the 2 study localities.*

Edward's Reef	Eagle Point
Mytilus edulis	*Mytilus californianus*
Littorina scutulata	*Balanus cariosus*
Acmaea digitalis	*Cucumaria pseudocurata*
Nereis vexillosa	*Thais canaliculata*
Gloiopeltis furcata (red alga)	*Thais emarginata*
	Acmaea pelta
	Acmaea digitalis
	Acmaea ochracea
	Onsidiella sp.
	Ligia pallasii
	Hemigrapsus nudus
	Ishnochitin mertensii
	Tubulanus polymorpha
	Syllus sp.
	Leptasterias hexactis

and 2) fresh water run-off. The freshwater is collected in low meadows and runs into a deep trench which leads to a high tidal pool above the *P. polymerus* clusters studied at Edward's Reef. The fresh water trickles over the barnacles during all but the warmest months. On April 27th and 28th, 1974, sea water samples were taken and salinity calculated ($8^{\circ}C$);

Edward's Reef, ocean	30.3 0/00
Edward's Reef, high tidal pool	12.2 0/00
Eagle Point, ocean	30.3 0/00

Bays tend to provide shelter from waves, whereas headlands tend to concentrate the wave energy on themselves (Tricker, 1965, p. 96). There is a turbulent convergence point of current activity off of Eagle Point while no such phenomenon occurs at Edward's Reef. Southeast and southwest winds are also more prominent at Eagle Point (C. Vandersluys, Personal communication). Perhaps, due to the combination of these forces, the currents bring in an abundance of plankton and small fish to Eagle Point which draw salmon runs during the spring and summer (Personal observation). Since there is apparently not as much plankton available at Edward's Reef, salmon are not fished in this area.

Fecundity. There is no correlation of embryo mass dry weight with embryo stage (r = -0.004 to -0.017) or with parental size (r = 0.028 to 0.119) when the calculated values are compared with values for r required for significance (Steele and Torrie, 1960, p. 453).

Since there is no correlation of egg mass weight with adult size, egg masses may be grouped and their means compared. There is a significant difference when localities are compared ($p < .001$), but not when intertidal height within locality or when month (May to August) or interaction of month and locality are compared. It is clear, then, that the amount of embryos produced in any 1 brood by an adult depends not upon the time of the reproductive season, but only upon location of the adult, and therefore probably upon some environmental factor(s). When the average weights of egg masses produced over most of the reproductive period are compared for the 4 localities, the same trend as previously observed for adult size, juvenile growth rate and percent brooding is indicated (Table 6): greater reproductive success at low than at high tidal levels, and greater success at Eagle Point than at Edward's Reef.

Fecundity was compared in the 4 populations by estimating the number of broods or embryos produced per adult per season (Table 7). The method of estimation was adapted from Hilgard (1960): $A/B \times D/25 = y$, where y is the maximum number of broods produced per mature adult per reproductive season, A is the total number of mature adults brooding during the reproductive season over the 26 months sampled, B is the total number of mature adults sampled during the reproductive season over 26 months, D is the total number of days during the reproductive season, estimating the beginning and end from brooded embryo stage data: 1) a full month if more than a single stage (early, middle and late) is represented or 2) a half month if only a single stage is represented (Figs. 16 and 17), and 25 is the mean number of days taken for embryos to develop from fertilization to hatching *in vitro* (Lewis, 1975b). A/B is, thus, the proportion brooding during the sampling period. Again, the trend is for Eagle Point low animals to produce the most broods per season and animals at Edward's Reef high to produce the fewest.

Table 6. *Mean dry weight (g) of embryo mass per adult* Pollicipes polymerus *at the 4 study localities.*

Date	Eagle Point		Edward's Reef	
	High	Low	High	Low
May	.0649	.0712	.0000	.0000
	.0910	.0984	.0524	.0640
June	.0538	.0794	.0064	.0237
	.0442	.0571	.0142	.0439
July	.0927	.1028	.0376	.0450
	.0266	.0932	.0201	.0429
	.0354	.0602	.0115	.0187
August	.0690	.0760	.0443	.0751
	.0510	.0468	.0304	.0234
	.0349	.0339	.0095	.0168
Mean	.0563	.0719	.0226	.0353

Table 7. *Comparison of fecundity in 4 populations of* Pollicipes polymerus. *(See text for explanation).*

Locality		Percent brooding	Estimated no. days brooding in a reproductive season	No. broods produced per reproductive season
Edward's Reef	high	41.0	120	2.0
	low	60.3	120	2.9
Eagle Point	high	56.6	180	4.1
	low	56.8	195	4.4
Mean		53.4	154	3.3

Discussion

Growth

The slow growth rate and relatively long time to sexual maturity determined for *P. polymerus* in this study agree with that for *P. spinosus* (Batham, 1945) and extend the data of Barnes and Reese (1960) and Paine (1974) for *P. polymerus*. Paine (1974) found juveniles growing 0.7 to 1.2 mm RC/month in northern Washington, while growth rates ranged from 0.2 to 1.0 mm RC/month on San Juan Island. Adults also grew slightly faster at Paine's study sites (4.2 mm RC/year) than on San Juan Island (1 to 2 mm RC/year), possibly due to the open coastal environment providing more food and/or stimulating feeding activity more. However, mean growth rate was measured in *P. spinosus* at an unprotected rocky site in New Zealand over 4 and one-half years: 1.0 mm/year in juveniles and 0.8 mm/year in adults (Batham, 1945). These growth rates are slower than those of *P. polymerus* at San Juan Island.

The growth rate of the young spat during the first year after spring or summer settlement is rapid and then slows down considerably. Growth may be seasonal. It is slower in the cold months than in the warm months for *B. balanoides*, *B. balanus*, *B. crenatus* and *Verruca stroemia* (Barnes, 1952-53, 1955b, 1958; Barnes and Powell, 1953; Barnes and Barnes, 1954). Seasonal growth trends are likely due to regionally varying factors, such as temperature and food availability (Crisp, 1960). It is also possible that the wearing away of capitulum plates (which are measured for size) occurs seasonally, thus giving a false impression of growth rates.

Two environmental factors which influence food availability, and thus growth rate, in other species are: 1) tidal level (Hatton and Fischer-Piette, 1932; Moore, 1934a; Barnes and Powell, 1953) and 2) differing exposures to wave action (Hatton and Fischer-Piette, 1932; Moore, 1934a; Newcombe, 1935; Hatton, 1938; Moore and Kitching, 1939). These factors may affect *P. polymerus* juvenile growth rate and adult size.

Reproductive Biology

Size at sexual maturity. It is apparent from Figures 9 and 12 that the adults at Edward's Reef are consistently smaller than those at Eagle Point. Juveniles also grow more slowly at Edward's Reef than at Eagle Point and at high intertidal levels at both locations so that smaller does not necessarily mean younger animals. Crisp and Patel (1961) found that crowding slows growth and delays ovary maturation in *Elminius modestus*, such that very slow growing individuals mature at a small size. It is possible that growth is slowed due to a smaller amount of food available at Edward's Reef, and that gonad maturation is delayed.

Animals measuring from 10 to 34 mm RC were found brooding embryos at San Juan Island. These results agree for the most part with those of Hilgard (1960), although she did not find mature animals smaller than 17.2 mm at Monterey, California. The majority of the brooding population was between 17 and 32.5 mm at Eagle Point and between 14 and 27.5 mm at Edward's Reef.

Reproductive cycle: local variation. In the 2 years sampled, *P. polymerus* at Eagle Point began to brood embryos in April or May;

brooding began in late May or June at Edward's Reef (Fig. 18). Adults at Edward's Reef were just starting to brood in June, 1972, while adults at Eagle Point had almost reached their brooding peak. By contrast, in June, 1973, brooding at Edward's Reef had reached its peak before the peak of brooding at Eagle Point. During July, 1971, sampling began at the peak of brooding, which in that year seemed to coincide at the 2 localities. Similarly, in July, 1972, the peaks coincided, but in July, 1973, statistically significant differences between the 2 localities were observed.

There is greater wave action at Eagle Point than at Edward's Reef. The low intertidal animals at both localities and the animals at Eagle Point have the advantage of longer feeding periods. In addition, fresh water run-off occurs for most of the year at Edward's Reef (but not at Eagle Point). Since *P. polymerus* is an osmoconformer which tolerates sea water dilutions down to about 50%, (Fyhn *et al.*, 1972), excessive fresh water might cause physiological stress so that these animals would have less energy for gametogenesis.

Reproductive cycle: latitudinal differences. The brooding season in *P. polymerus* is from April to October at San Juan Island, Washington (present study); the end of March or beginning of April to the end of December or beginning of January near San Francisco, California (Hand *et al.*, 1973); April to December at Monterey Bay, California (Hilgard, 1960); and presumably all year near Santa Barbara, California (Straughan, 1971). The peak of brooding occurs during February at Santa Barbara (Straughan, 1971), compared with July at San Juan Island and August at Bodega Head. The time of first brooding is not delayed in the northern populations. Instead, the brooding season continues for longer periods as one moves south. Straughan (1971) also noted that *P. polymerus* is less abundant, smaller, and that fewer brood south of Santa Barbara than in the Santa Barbara Channel and north.

Reproductive cycle: effects of photoperiod and temperature. Although the factors inducing breeding in winter breeders are fairly well understood, they are not well known for summer breeders. As with other summer breeders (Table 8), the reproductive cycle of *P. polymerus* at San Juan Island may be correlated with day length and temperature.

Latitudinal differences are probably a reflection of a critical air and/or sea water temperature range beyond which breeding is not physiologically feasible. Since the annual air temperature fluctuation (-10° to +30°C, *Friday Harbor Journal* for 1972-73) is greater than the variation in surface sea water temperature (6.2° to 13.6°C, Johnson and Thompson for 1927-28) at San Juan Island, mid-intertidal species such as *P. polymerus* may be affected as much or more by air temperature. Brooding occurs at mean monthly sea water temperature ranges which overlap. The peak of the reproductive period occurs when the sea water temperature is about 11°C at San Juan Island and San Francisco, while it is about 14°C at Monterey and Santa Barbara. Brooding of *P. spinosus* in New Zealand (44° S) (Batham, 1945) occurs from mean sea water temperatures of 9° to 15°C and peaks at 14°C; similar to that for *P. polymerus* at San Francisco (38° N) in temperature range during brooding, but closer to that for Monterey (36° N) or Santa Barbara (34° N) for sea water temperature at the peak of brooding. The cycle of reproduction of *P. spinosus* is opposite to the cycle of *P. polymerus* at Monterey Bay north to San Juan Island, but parallels the cycle of *P. polymerus* at Santa Barbara.

Table 8. *Comparison of reproductive data on breeding period for various cirripedes.*

Species	Locality	Breeding Period	Reference
Balanus balanoides	Atlantic coast of Canada	Oct-Apr	Bousfield, 1952-3
Balanus balanoides	Wood's Hole, Mass.	Dec-May*	Bousfield, 1952-3
Balanus balanoides	British Isles	late fall-spring	Crisp, 1958a
Balanus balanoides	British Isles	Nov-Mar	Moore, 1935b
Balanus balanoides	Millport, Scotland	Aug-Nov	Pyefinch, 1948a
Balanus glandula	La Jolla, Calif.	Oct-Dec. Mar-May	Barnes & Barnes, 1956b
Balanus glandula	San Francisco, Calif.	late Feb-Jun	Herz, 1933
Balanus glandula	Vancouver Island, British Columbia	Jan-Mar	Barnes & Barnes, 1956b
Balanus tintinnabulum	La Jolla, Calif.	all year*	Coe, 1932
Balanus crenatus	San Francisco Bay, Calif.	Feb-Nov	Herz, 1933

Table 8. (Continued)

Species	Locality	Breeding Period	Reference
Balanus crenatus	Gulf of Maine	Apr-Jun* Sept-Oct*	Bousfield, 1952-3
Balanus crenatus	British Isles; Wales	Jan-May	Crisp & Patel, 1969
Balanus crenatus	north of Wales, U.K.	Jan-Sept	Crisp & Patel, 1969
Balanus crenatus	Millport, Scotland	Feb-Aug	Pyefinch, 1948a
Balanus improvisus	Japan Sea	Mar-late Apr	Yasuda, 1970
Balanus improvisus	west coast of Sweden	Apr-Dec	Blom, 1965
Balanus improvisus	Gulf of St. Lawrence	Jun-Jul* Sept-Oct*	Bousfield, 1952-3
Balanus balanus	British Isles	Feb-Nov	Crisp & Patel, 1969
Balanus balanus	British Isles	mid-winter to spring	Barnes, 1963b
Balanus balanus	Atlantic coast of Canada	Mar-Jun*	Bousfield, 1952-3

Table 8. (Continued)

Species	Locality	Breeding Period	Reference
Balanus hameri	British Isles	Dec-Mar*	Moore, 1934b
Balanus hameri	British Isles	early Jan-early Mar	Crisp, 1954
Balanus hameri	Atlantic coast of Canada	Apr-Jun	Bousfield, 1952-3
Balanus amphitrite	Calicut, India	all year*	George, 1958
Balanus amphitrite	east coast, Madras, India	all year	Daniel, 1954
Balanus amphitrite	Vizag, India	all year	Ganapathi *et al.*, 1958
Balanus amphitrite	open coast, India	all year	Pillai, 1958
Balanus amphitrite	Cochin Harbor, India	Nov-Apr	John, 1964
Balanus amphitrite	Narackal Harbor India	Dec-Jun*	George, 1958
Balanus trigonus	Japan Sea	May-end July; Sept-late Nov	Yasuda, 1970

Table 8. (Continued)

Species	Locality	Breeding Period	Reference
Balanus venustus	Japan Sea	late Apr-late Jun	Yasuda, 1970
Balanus eburneus	Japan Sea	Jul-Sept.	Yasuda, 1970
Elminius modestus	British Isles	all year	Crisp & Davies, 1955
Elminius modestus	British Isles	all summer	Crisp & Chipperfield, 1948
Elminius modestus	Menai Bridge	all summer	Crisp & Patel, 1961
Chthamalus stellatus	Brixham, U.K.	low water: Apr-Sept high water: May-Sept	Crisp, 1950
Chthamalus stellatus	North Wales	Apr-Sept	Crisp, 1950
Chthamalus stellatus	Scotland	May-Sept	Crisp, 1950
Chthamalus challengeri	Asamushi, Japan	Mar-Oct	Luckens, 1968
Verruca stroemia	British Isles	all year*	Pyefinch, 1948a

Table 8. (Continued)

Species	Locality	Breeding Period	Reference
Pollicipes spinosus	Dunedin, New Zealand	Dec-Jun	Batham, 1945
Pollicipes polymerus	Goleta Point, Calif.	all year	Straughan, 1971
Pollicipes polymerus	Monterey Bay, Calif.	May-Dec	Hilgard, 1960
Pollicipes polymerus	Duxbury Reef, Calif.	Mar-Jan; Apr Dec	Hand *et al.*, 1973
Pollicipes polymerus	Bodega Head, Calif.	Apr-Dec	Hand *et al.*, 1973
Pollicipes polymerus	Eagle Point, San Juan Island	May-?	Fyhn *et al.*, 1972; Petersen *et al.*, 1974
Pollicipes polymerus	Eagle Point, San Juan Island	low water: Apr-Oct high water: May-Oct	Present study
Pollicipes polymerus	Edward's Reef, San Juan Island	low water: May-Aug high water: May-Sept	Present study

* Reported as season during which larvae are liberated.

In the winter breeder *B. balanoides* (Table 8), Barnes (1963a), Barnes and Barnes (1967), and Crisp and Patel (1969) showed that continuous illumination delays the onset of breeding, inhibits male and female gonadal development, and inhibits the cyclic growth of the oviducal gland and penis. Barnes and Stone (1972) and Tighe-Ford (1967) demonstrated that less than 12 h of light per day was necessary for stimulation of breeding 4 to 8 weeks later.

Crisp (1957), Barnes (1963a), Barnes and Barnes (1967), Tighe-Ford (1967) and Crisp and Patel (1969) showed an upper critical temperature exists above which breeding will not occur in *B. balanoides*. Barnes and Barnes (1956b) found that *B. glandula* breeds only below 16° to 17°C throughout its distribution. Crisp (1957) induced breeding in *B. balanoides* and *B. balanus* with low temperatures, while Crisp and Patel (1969) also induced *B. balanus* and *B. crenatus* to breed prematurely by lowering the temperature. Crisp and Clegg (1960) showed that constancy of the start of the reproductive period could not depend on annual temperatures in *B. balanoides*, but it is possible that long term temperature and photoperiod values jointly determine the date. Crisp and Patel (1969) were unable to induce premature breeding in *B. balanoides* with low temperature. They could do so by keeping individuals above a critical temperature to delay breeding and subsequently providing a conditioning period below the critical temperature.

Many cases of cyclic breeding activities in marine invertebrates have been considered a function of sea water temperature (Orton's Rule) (Appellof, 1912; Orton, 1920; Runnstrom, 1927; Hutchins, 1947; Bousfield, 1952-53). A good deal of data has been collected on a variety of animals which substantiates the temperature-breeding correlation. In his review of cyclic reproductive activity, Giese (1959) states that "growth and gamete maturation are probably influenced more by temperature than any other factor." Although there is no direct evidence that the onset of brooding in *P. polymerus* on San Juan Island is controlled by temperature, seasonal brooding trends follow sea water temperatures quite closely, as in *P. spinosus* (Batham, 1945).

Onset and cessation of brooding in *P. polymerus* takes place at approximately the same temperatures, even though these temperatures are not the same at Monterey Bay (Hilgard, 1960) as on San Juan Island. At Monterey Bay brooding begins and stops at 12° to 13°C, whereas brooding begins on San Juan Island at 8° to 9°C and stops at 9° to 10°C.

Although air temperature has not been discussed much in the literature as an influence on reproductive activity in marine invertebrates, it may be considered as an important environmental influence on *Pollicipes*, the only intertidal pedunculate barnacle. *P. polymerus* is usually an upper intertidal inhabitant and insolation has been shown to raise its body temperature above ambient levels (but stabilizes 2° to 5°C below rock temperature), whereas when submerged, *P. polymerus* assumes the temperature of the water (Fyhn *et al.*, 1972). It is possible that a critical minimum air temperature (e.g., 15°C) triggers breeding.

Shortening day length has a direct influence on the onset of breeding in the winter breeder *B. balanoides* (Tighe-Ford, 1967), but if photoperiod is causally related to the spring-summer breeding of *P. polymerus*, it must have the opposite effect.

Reproductive cycle: effects of food. The time of larval release has been shown in *B. balanoides* (Barnes, 1957) to be triggered by availability of food. Crisp and Patel (1969) found, however, that continuous feeding delayed the onset of breeding in *B. balanoides*.

One group of barnacles reproduces more or less continuously throughout the year (Table 8): *Elminius modestus* (Crisp and Davies, 1955) and *Verruca stroemia* in the British Isles (Pyefinch, 1948b) and *B. tintinnabulum* at La Jolla, California (Coe, 1932). Crisp and Davies (1955) found that regeneration of the ovary in *Elminius* depends upon the food supply.

Barnes (1963a) and Barnes *et al.* (1963) showed that gonad development in *B. balanus* occurs in the spring and early summer when phytoplankton is most plentiful. Patel and Crisp (1960a) found that the breeding rate in *C. stellatus*, *B. amphitrite* and *B. perforatus* depends entirely upon temperature and food supply.

P. polymerus is distributed primarily in latitudes where food is rarely limiting. However, the major phytoplankton bloom in the Puget Sound area occurs in the spring (Johnson and Thompson, 1929-31) corresponding to the time of brooding, and this may influence its onset.

Reproductive cycle: other environmental factors. Crisp (1959a) and Crisp and Clegg (1960) showed the date of fertilization in *B. balanoides* to be strongly influenced by the amount of algal cover, wave action and currents. Those animals exposed to strong currents fertilized much later than those in calm water; animals growing in exposed areas were fertilized later than those in sheltered locations. Barnes and Crisp (1956) noted that controls in reproduction experiments must be chosen with care since "tidal level, aspect and access to surf" can profoundly influence fecundity. Crisp and Davies (1955) showed that access to fast currents increased fecundity in *Elminius*, and that animals on the outer edge of a cluster had a higher proportion of brooding than those in the center. Blom (1965) showed the proportion of brooding in *B. improvisus* to depend upon population density.

Exposure to sewage and high residual chlorine concentrations at Monterey Bay reduced the numbers of adults brooding embryos, slowed gonad development, and reduced recruitment of *P. polymerus* (Holstrom, 1970); brooding being the most sensitive of these 3 estimates to pollution. There was also some evidence that fertilization was delayed due to the presence of sewage. Straughan (1971) observed large discrepancies in the proportion of adults brooding embryos (i.e., 10 to 90% in April, 1969) from oiled and non-oiled areas, respectively, near Santa Barbara, California. The percent of adults brooding embryos in oil-clean areas is twice that of adults in natural oil seep locations nearby. Statistically significant differences in percent of adults brooding was noted by Hand *et al.* (1973) between populations at Duxbury Reef affected by the San Francisco oil spill and those at Bodega Head which had not been affected. The percent of adults brooding at the unoiled site was higher than at either oiled site in May, August and September.

Even though *P. polymerus* has a brooding period on San Juan Island, local variation in reproductive activity occurs. Differences in brooding are observed on San Juan Island at locations about 5 miles apart. Thus, it is evident that environmental factors, e.g., wave action and fresh water run-off, may be operating on *P. polymerus*.

Fecundity. In most crustaceans the number of eggs produced is a function of the mass (or a linear size measurement) of the parent

(Barnes and Barnes, 1968). However, when *P. polymerus* size (RC length which is directly proportional to dry weight, Fig. 9) was compared to embryo mass dry weight, no correlation was found. Of course, weight of individual embryos may vary according to the conditions under which they were produced in *P. polymerus*. Numbers of embryos brooded per adult may also vary according to adult barnacle size, nutrient conditions and position on the shore (Barnes and Barnes, 1965), and latitude (Crisp, 1959).

The number of eggs produced in *B. balanoides* depends as much upon local conditions as upon latitude, the greatest number being produced under the most favorable habitat for feeding (Barnes and Barnes, 1968). The fecundity of *Chthamalus stellatus* (which feeds largely by extension of cirri like adult *P. polymerus*) is generally lower in protected areas, e.g., bays. It is suggested that low egg production in this species may be related with sand scouring. The same possibility may exist in the populations of *P. polymerus* at Doran Beach near San Francisco (Table 3). Since *P. polymerus* produces fewer eggs near the southern end of its range (Straughan, 1971), it may also occupy a suboptimal habitat there. Although Barnes and Reese (1960) felt that San Juan Island comprises a suboptimal habitat for *P. polymerus*, and Barnes and Barnes (1968) measured an extremely low weight of eggs produced at San Juan Island (per standard increment of body weight) relative to several species of acorn barnacles, this study shows that fecundity of the San Juan Island populations matches fecundity at Monterey Bay in the center of its range (Table 9). Although the San Juan Islands are semi-exposed, the fast currents, frequent storms, and upwelling bring with them an abundance of plankton.

Table 9. *Comparison of the fecundity of* Pollicipes polymerus *at different latitudes. (See text for explanation.)*

Location	Mean % brooding during reproductive months sampled	Duration of reproductive season (Days)	Peak(s) of reproductive activity (Month/%)	No. of broods	Estimated no. of embryos/ adult (range, x 10^3)	Estimated no. of embryos produced/ adult/ season (range, x 10^3)
San Juan Island, Washington 48° 30' N	53.4	195 April-Oct	July/77.4	4.2	144-288	605-1210
Bodega Head,* California 38° 19' N	27.9	270 April-Dec	Aug/61.9	3.0	—	—
Monterey Bay, California 36° 40' N	44.5	240 April-Dec	June/69.0 Sept/66.7 Dec/70.0	4.3	104-240	447-1032
Santa Barbara,* California 34° 20' N	55.0	365 All year	Feb/90.0	8.0	0.08-0.12	0.640-1.024

* Non-oil spill areas only.

Although a slight difference in the composition of *Pollicipes* communities at the 2 study localities was observed, it is concluded that primarily the amount of wave action (quantity of food available and aeration, Crisp, 1960) and perhaps, secondarily, intertidal height (length of feeding time) and fresh water run-off play important roles in regulating growth rate, adult size, reproductive activity, and fecundity.

When the number of broods produced at each latitude are compared (Table 9), it is noted that the fewest are produced at Bodega Head, slightly more at San Juan Island and Monterey Bay, and by far the most at Santa Barbara. However, only one-thousandth of the number of embryos are produced at Santa Barbara as at Monterey Bay or San Juan Island. The total reproductive output has, thus, suffered at the southern end of the species' range.

When Hilgard (1960) compared fecundity in *P. polymerus* (Monterey Bay) and *P. spinosus* (New Zealand, Batham, 1945), she found that *P. polymerus* produces about 57 times the number of embryos per brood as *P. spinosus*. If we further compare fecundity (expressed here as the mean number of eggs produced per 25 mg dry body weight of a medium-sized animal) in *P. polymerus* at San Juan Island (2400 eggs) with that of *P. cornucopia* at Cabo Silleiro, Gilbralter (15,400 eggs, Barnes and Barnes, 1968), we find about 7 times as many eggs produced per brood by the European species. Since the weight of individual embryos in these 3 species is not known, one cannot compare the dry weight of embryos produced. Embryos in different species likely differ in size and/or weight; e.g., *P. spinosus* produces large, yolky eggs while the eggs of *P. polymerus* are smaller and less yolky. Therefore, intraspecific comparisons of fecundity are probably more meaningful than interspecific comparisons when one is limited to the present data.

Copulation. It is not known if the daily decrease of tidal height during a monthly tidal cycle specifically affects breeding. It is likely that more "acting females" will be physiologically ready (with mature ova and well-developed oviducal glands) for breeding when previously restrained from breeding by lowering tidal levels. Thus, when the tide finally comes in, it may trigger a relatively high percentage of copulation.

No external factors that were manipulated in this study successfully stimulated copulation in *P. polymerus*. Although copulation has been observed in the field for *B. glandula* during an incoming tide (Wong, 1967), and for *B. balanoides* (Walley *et al.*, 1971) in the laboratory when resubmerged after several hours out of water, experiments of this type with *P. polymerus* produced no copulation.

Wong (1967) observed "clearly directional probing" by the penis in *B. glandula* and *B. tintinnabulum* as did Barnes and Barnes (1956a) for *B. balanoides*. Wong (1967) also noted multiple copulations with 1 receiver in *B. glandula*. These observations suggest that a receiver may produce an attractant. Collier *et al.* (1956) reported that treatment with ascorbic acid produced searching and copulatory activity in some balanoids and Barnes and Finlayson (1962) subsequently found that the semen of *B. balanus* contains much ascorbic acid. In only 1 case was any activity in *P. polymerus* noted: 2 barnacles were found extruding sperm and harboring ovulated eggs, but no copulatory activity was observed.

Wong (1967) observed that insemination stimulated ovulation in *B. glandula* and *B. tintinnabulum* and Walley *et al.* (1971) noted the same in *B. balanoides*. It is possible that ova or oviducal glands produce some chemical attractant which stimulates mating behavior and insemination. This behavior would then stimulate ovulation. However, neither crushed, ripe ova, nor ascorbic acid stimulated mating in *B. glandula* (Wong, 1967).

Barnes and Barnes (1956a) observed that most barnacles molt after fertilization and have suggested that molting may be intimately related to fertilization. It has been suggested that a basic physiological rhythm controls molting in *B. balanoides* (Crisp and Patel, 1960).

A number of conditions which tend to advance the fertilization date in *B. balanoides* (high intertidal level, little water currents, low light intensity, low temperature and short day length) slow down the metabolic rate, suggesting a common mechanism (Crisp, 1959a). Likewise, from the environmental data recorded at San Juan Island, an increase in metabolic rate may influence onset and intensity of brooding in *P. polymerus* (low intertidal level, strong water currents, high temperature and long day length).

Cross-fertilization is common in hermaphroditic barnacles. Copulatory activity and insemination have been described for *B. glandula* and *Chthamalus dalli* (Wong, 1967), for *B. balanoides* (Barnes and Barnes, 1956a; Clegg, 1957; Walley *et al.*, 1971) and for *B. balanus* (Crisp, 1954). Obligatory cross-fertilization is likely for *B. balanoides* (Crisp, 1950; Crisp and Patel, 1960), *B. crenatus, Elminius modestus* (Crisp, 1950, 1954, 1958b) and *B. balanus* (Barnes and Barnes, 1954; Crisp, 1954). Some barnacles, however, indulge in self-fertilization (Barnes and Crisp, 1956), since experimentally isolated *Chthamalus stellatus, Verruca stroemia* and *B. perforatus* produce embryos. These species probably cross-fertilize most often in the field, but are facultative self-fertilizers, with breeding being slightly delayed from the normal period when isolated. Barnes and Crisp (1956) noted that self-fertilized eggs were frequently less viable than cross-fertilized eggs in the same species.

P. polymerus of reproductively mature size apparently do not brood embryos when they are separated by more than 11 cm at Eagle Point. Hilgard (1960) showed that this species broods when isolated by up to 20 cm, but no farther, at Monterey Bay, California. This distance probably depends upon penis length, and thus, adult size.

Individuals in populations of *P. polymerus* are very dense, and are thus, well-adapted for cross-fertilization. Settlement is gregarious with cyprids preferring adult peduncles (Lewis, 1975b). When operculates show such behavior, it is largely to ensure the proximity required for obligatory cross-fertilization. However, Barnes and Reese (1960) stated, "There is evidence . . . that in *Pollicipes polymerus* self-fertilization is the rule . . . ." It is unfortunate that this evidence is not presented for scrutiny. They argue that since *Pollicipes* is a primitive genus, self-fertilization may be an "evolutionary relict" or is secondarily derived. They suggest that aggregation protects the young and that settlement on adult peduncles may be a carryover from an ancestral subtidal species living where few good settling surfaces were available.

Since copulation has not been witnessed, it is impossible to conclude whether *P. polymerus* cross- or self-fertilizes. However, since isolated individuals do not reproduce (Fig. 13; see also Hilgard, 1960) cross-fertilization may normally occur in *P. polymerus*, as it does in most other species of free-living barnacles (Clegg, 1957; Wong, 1967).

SOME OBSERVATIONS ON FACTORS AFFECTING EMBRYONIC AND LARVAL GROWTH *IN VITRO*[1]

Introduction

Batham (1946) first raised barnacle embryos (*Pollicipes spinosus*) through to settling *in vitro*. That species produces yolky eggs which develop directly. Crisp (1959b) was the first to culture eggs which are less yolky and develop indirectly. Other attempts to culture embryos have been made with varying degrees of success (Barnes, 1957; Barnes and Barnes, 1959a; Patel and Crisp, 1960b), but few experimental studies have been performed with cultured embryos. Results to date have shown that temperature influences developmental time (Crisp and Davies, 1955; Barnes and Barnes, 1959a; Crisp, 1959b; Patel, 1959; Patel and Crisp, 1960b; Crisp and Costlow, 1963; Blom, 1965) and probably influences embryo size as well (Patel, 1959; Patel and Crisp, 1960b).

Using various techniques, workers have also cultured nauplii in the laboratory, some with success (Yasugi, 1937; Costlow and Bookhout, 1958; Moyse, 1961; Hirano, 1962; Freiberger and Cologer, 1966; Tighe-Ford *et al.*, 1970; Molenock and Gomez, 1972; Karande, 1974). Crisp (1962) and Snodgrass (1967) were less successful. See reviews by Costlow and Bookhout (1957), Bookhout and Costlow (1959) and Moyse (1963) for information.

Some variation of naupliar size within each stage has been found in most barnacles (Knight-Jones and Waugh, 1949; Norris and Crisp, 1953; Jones and Crisp, 1954; Costlow and Bookhout, 1957, 1958; Moyse, 1961; Molenock and Gomez, 1972). Extreme variability in size (Sandison,

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[1] In press in *Marine Biology*

1967; Tighe-Ford *et al.*, 1970) as well as variability in the oldest larval stages reached (Moyse, 1963) may reflect the effect of less-than-optimal temperature and light conditions and food concentration during naupliar growth.

Natural supplies of barnacle larvae are impractical for experimental uses since their availability may be seasonal and sorting specimens from mixed populations is time consuming. With cultures being the most practical source of larvae, it is desirable to identify the factors that affect growth and development. Furthermore, most reports of developmental timetables are not accompanied by detailed descriptions of the methods employed to determine those results. Thus, comparison of results from study to study is very difficult.

In this paper, observations on the effects of antimicrobial drugs commonly added to cultures, illumination, aeration, embryo mass size, foods given to nauplii, and food density are reported for *Pollicipes polymerus* (Sowerby, 1833), a very common intertidal barnacle on the West Coast of North America. The data were collected during a study in which the barnacles were reared from gastrulation through settlement.

Materials and Methods

Culture of Embryos

Adult *Pollicipes polymerus* were collected from the shore and dissected for lamellae (egg masses) within 2 h. These lamellae were immediately divided into experimental groups. Control lamellae were placed in 200 cm^3 glass finger bowls with autoclaved and filtered sea water, natural sunlight from laboratory windows of north and south exposure (April to June, about 13 to 16 h light per day), and no drugs.

The water was changed and embryos were sampled every 2 to 3 days. Variables introduced were: natural lighting or total darkness (as in the adult barnacle mantle cavity); aeration by constantly bubbling air through air stones into individual finger bowls (as in turbulent water, where there is much wave action) or no aeration; treatment with full and half-strength streptomycin, penicillin and chloromycetin (full strength as in Barnes and Barnes, 1959; Tighe-Ford *et al.*, 1970) or no treatment; flowing sea water or still water; and Pyrex vessels of varying sizes (100 to 1000 cm^3). Three replicates of each of the 26 culture conditions were used. Samples were taken by cutting off a tiny piece of the outer lamella with glass needles and fixing in 95% ethanol so that embryos could be separated from one another and examined microscopically to determine the stage of development and to detect morphological anomalies. The containers were scrubbed and rinsed with tap water at each water change and embryo sampling period.

Culture of Larvae

Both single and multiple factor experimental designs were used to evaluate the effects of food types and food concentration individually, and in combination, on larval survival and development. A preliminary experiment was performed to determine the food types in which the best growth and molting rates could be found. The next experiment was designed to monitor growth differences between larvae fed different algae and differences between larvae fed 2 algal concentrations.

Pollicipes polymerus lamellae in which nauplii appeared ready to hatch (Lewis, 1975b) were freshly collected and pooled in large finger bowls of sea water. The positively phototrophic Stage 1 nauplii hatched out from the egg masses and collected in a narrow beam of light. Only those nauplii which swam into the light beam within 30 min were used for experiments.

Sea water was pumped to the laboratories through systems made of inert materials. The water was Millipore-filtered, removing particles as small as 3 μ, and ultra-violet light-treated for cultures. Antibiotic solutions of Penicillin G and streptomycin sulphate were added (concentrations as in Tighe-Ford *et al.*, 1970) when the water was changed. Water was collected every 2 to 3 days in 5-gallon (19-L) carboys and stored at 12° to 13°C. The preliminary experiments were conducted at the Friday Harbor Laboratories (ambient sea water temperature 12° to 13°C, spring) and the later experiments at the Bodega Bay Marine Laboratories (ambient sea water temperature 13° to 16°C, summer). All larval and algal cultures were continuously illuminated overhead by 20 W fluorescent tubes in an incubator for optimum conditions and maximum algal growth (Tighe-Ford *et al.*, 1970).

Preliminary Screening of Foods and Culture Conditions

Various algae were cultured on diatom Medium F/2 (Guillard and Ryther, 1962) in nonsterile, unialgal culture and these were fed to *P. polymerus* larvae so that a growth-stimulating food could be found. The following species were tested: *Phaeodactylum tricornutum*,[2] *Platymonas suecia*[2] and *Platymonas* sp.,[3] *Isochrysis galbana*,[2,3] *Skeletonema costatum*,[2] *Prorocentrum micans*,[3] *Chrysochromulina* sp.,[3] *Micromonas pusilla*,[3] *Chaetoceros* sp.,[3] *Cryptomonas* sp.,[3] *Amphiprora* sp.,[3] *Ditylum* sp.,[4]

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[2] Primary culture from Dr. J. Lewin, Oceanography Department, University of Washington. [3] Primary culture from Dr. R. Norris, Botany Department, University of Washington. [4] Primary culture from Bodega Bay Marine Station Collection.

Hymenomonas carterae,[4] *Amphidinium* sp.,[4] a small centric diatom,[5] a pennate diatom and combinations thereof. Algae were maintained in 1-L Erlenmeyer flasks and thinned by diluting with fresh medium when they became too dense. Larvae were grown singly in spot-plate wells and were monitored twice daily through the dissecting microscope for activity, general state of health, developmental stage, and amount of food in the gut (opaque, full; translucent, partly full; clear, empty). Exuviae were collected and preserved in 70% ethanol. Five replicates of each culture condition were made.

Experiments showed that a large proportion of nauplii died at Stage 2 when fed most of these algae. Several negative effects on the larvae by algal foods were observed (Table 10). The culture conditions in which larvae lived beyond Stage 2 are presented in Tables 10 and 11.

Larvae fed *Prorocentrum micans* and *Prorocentrum micans/Platymonas* sp. reached Stage 3 twice as fast at 17°C as at 12° and 13°C (Table 11). The larvae grown at the higher temperature were also without antibiotics. At 17°C no larvae grew beyond Stage 3. Although it was impossible to determine which of these 2 factors was responsible for larval death, the decision was made to use ambient temperature sea water (as above) and to add antibiotics in later experiments.

Food Type and Concentration Experiments

The larvae were reared as batches of 125 (0.5 larvae/cm^3) and 450 (1.8 larvae/cm^3) in 0.5-L Pyrex beakers initially prepared in cleaning solution. Black tape was attached around the outside of the

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[5] Primary culture from M. Landry, Graduate Student, Oceanography Department, University of Washington.

Table 10. Pollicipes polymerus. *Results of feeding nauplii different algae.*

Food	Latest stage reached	Observed Results
Skeletonema costatum	2	Algae stick to larval appendages; larvae entrapped and use up much energy attempting to escape.
Cryptomonas sp.	2	
Centric diatom	2	
Chaetoceros sp.	2	
Amphiprora sp.	2	
Ditylum sp.	2	
Hymenomonas carterae	2	Algae ingested; larvae lose motility, become lethargic and weakened.
Amphidinium sp.	2	
Chrysochromulina sp.	2	
Isochrysis galbana	4	
Phaeodactylum tricornutum	2	Algae ingested; larvae retain motility, but do not develop.
Pennate diatom	5	Algae ingested; larvae unable to molt normally; larvae retain exuviae.
Platymonas sp.	short-lived cyprid	Algae ingested; larvae healthy, motile.
Prorocentrum micans	cyprid	

Table 11. Pollicipes polymerus. *Mean number of days from hatching to Naupliar Stages 2 through 6 from a representative run. Nauplii in Group A were kept at 12° to 13°C with antibiotics, nauplii in Group B at 17°C with no antibiotics. Algae fed in combinations were added in the ratio of 1:1 (v/v). n is the number of specimens observed.*

Group	Food	Naupliar Stage									
			2		3		4		5		6
		n		n		n		n		n	
A	*Platymonas* sp.	5	1.0	5	9.0	2	18.0	2	24.5	1	39.0
	Prorocentrum micans	5	1.0	5	8.6	5	13.0	1	22.0		
	Platymonas sp. + *Prorocentrum micans*	4	1.0	4	8.0	3	13.0	2	22.0		
	Diatom (unknown species)	4	1.0	2	6.5	2	12.5				
	Platymonas sp. + *Prorocentrum micans* + *Phaeodactylum tricornutum*	1	1.0	1	10.0	1	16.0				
	Isochrysis galbana	1	1.0	1	10.0	1	16.0				
	Isochrysis galbana + *Prorocentrum micans*	1	1.0	1	7.0	1	12.0				
	Platymonas sp. + *Phaeodactylum tricornutum*	4	1.0	4	9.7						
B	*Prorocentrum micans*	3	1.0	3	4.0						
	Platymonas sp. + *Prorocentrum micans*	1	1.0	1	4.0						
	Diatom (unknown species)	1	1.0	1	5.0						

beakers at the meniscus level to avoid naupliar concentration and stranding on the vessel's sides. Beaker tops were also covered with black tape so that larvae were provided with diffuse light (Tighe-Ford *et al.*, 1970). The rearing vessels contained 250 cm^3 of cultured algae. Initial algal densities were calculated from the algal density-size relationship given by Moyse (1963). These densities were kept constant (Table 12) by using a hemocytometer for calculating all concentrations when changing the water and food. *Prorocentrum micans* was used in half the concentration (5 cells/mm^3) in the mixed food cultures as in the pure *Prorocentrum micans*-fed larval cultures (10 cells/mm^3). Due to slow algal growth, the concentration in the other cultures fed *Prorocentrum micans* was also reduced to 5 cells/mm^3 on Day 27. On Day 30, the *Platymonas* sp. concentration was increased from 500 to 1000 cells/mm^3 in the pure *Platymonas* sp.-fed larval cultures to determine if an increase in growth rate would result.

Replicates of each culture situation were run. The larvae were sampled and examined every 2 to 3 days. Filters with 73 and 130 μ meshes were used to capture early and later stages of nauplii, respectively, when changing the water. About 4% of the initial larval population was removed and placed in 95% ethanol at each change.

Results

Factors Affecting Embryonic Development in vitro

The effects of 4 categories of environmental factors were studied: 1) natural illumination or complete darkness; 2) whole or half lamellae; 3) aeration or no aeration; 4) 6 different drug combinations. To

Table 12. Pollicipes polymerus. *The algae fed to nauplii which resulted in best growth. A: approximate size of individual cells* (μ); *B: approximate no. of cells/mm^3 in rearing vessels; C: approximate no. of cells in each rearing vessel.*

Algae used	A	B	C
Prorocentrum micans	20 x 50	10	2.50 x 10^6
Prorocentrum micans (in combination with other algae)	20 x 50	5	1.25 x 10^6
Platymonas sp.	12-15	500	125.00 x 10^6
Pennate diatom	5-6	1000 (clumps)	250.00 x 10^6

determine the effect of 1 condition in a multiple factor experiment, pairs of test samples differing in but 1 variable were contrasted.

Darkness, aeration and small lamella size promoted faster embryonic growth (Table 13). Addition of full- or half-strength drugs shortened the average development time from labrum formation until hatching by 0.4 to 2.3 days. In all cases but 1, full-strength drugs increased growth rate in comparison to half-strength drugs, and half-strength drugs increased growth rate in comparison to no drugs. Full-strength compared to half-strength dosage tests were pooled and averaged, and the same was done with half-strength drugs compared to tests without drugs. Developmental rate was increased over the control embryos by an average of 0.2 days when the first half dosage of drugs was given, and by an average of 1.3 days with the second half. The second dosage was more effective than the first, possibly indicating a cumulative or threshold effect.

Abnormal embryonic development was determined by 1) asynchronous organ development compared to the normal pattern, and 2) lack of larval hatching although the egg mass was fragmenting. Embryos cultured with full-strength drugs developed abnormally more often than those with half-strength or no drugs. Embryos retained in whole lamellae and kept in natural illumination appeared more susceptible to factors causing abnormal development than those in half lamellae kept in the dark. Aeration did not affect normality of development. Embryos cultured in different sized vessels developed at the same rate. Embryos cultured in running water developed more blue-green algae and diatom epifaunal growth than those in still water.

Table 13. Pollicipes polymerus. *Mean embryonic development time (days) from formation of labrum to naupliar hatching and mean difference in time (days), comparing 2 factors at a time.*

Condition	No. of specimens	Development time (days)	Time difference (days)
Light/dark	22/22	10.5/9.7	0.8
Whole/half lamellae	23/23	10.2/10.0	0.2
No aeration/aeration	30/16	10.5/9.2	1.3
Half/full-strength penicillin and streptomycin	4/8	9.8/9.2	0.6
Half/full-strength chloromycetin	4/8	11.8/9.5	2.3
Half/full-strength penicillin, streptomycin and chloromycetin	4/8	10.5/9.6	0.9
Half-strength penicillin and streptomycin/no drugs	4/10	9.8/10.9	1.1
Half-strength penicillin, streptomycin and chloromycetin/no drugs	4/10	10.5/10.9	0.4
No drugs/half-strength chloromycetin	10/4	10.9/11.8	0.9

Effects of Food Type on Larval Development

Five feeding regimes were employed in the larval experiments: *Platymonas* sp. and *Prorocentrum micans*, separately and together, and a pennate diatom (unknown species) alone and with *Prorocentrum micans*. The average larval sizes were very similar up to 6 days after hatching, at which time larvae eating *Prorocentrum micans/Platymonas* sp. began to grow faster than the other larvae, and nauplii eating the diatom began to grow slower (Figs. 19 and 20). The correlation coefficients between larval stage and time were high in all cases except when larvae were fed the *Prorocentrum micans*/diatom diet (r = 0.34, Table 14). Therefore, regression lines were plotted for the 6 remaining cases. The coefficient of determination (r^2) shows the amount of variation in the stages reached by larvae due to time (i.e., growth) alone. The r^2 value was high (above 70%) except for larvae eating *Prorocentrum micans*/diatom which died 18 days after hatching, and for larvae eating *P. micans* alone (Table 14).

Larvae fed *Prorocentrum micans/Platymonas* sp. grew to Stage 6 faster (from Day 9 through Day 30) than larvae fed either alga alone or any other alga (Figs. 19 and 20; Table 14). Indications that the combination food enhanced larval growth to Naupliar Stage 6 more than either component alga were: 1) the average stage value for the combination food was greater than for either component (Table 14); 2) less variation in growth rate was due to time in individuals eating either food separately than in individuals eating the combination food (r^2, Table 14); 3) larvae grew faster on the combination food than on either component food (predicted average time, Table 15). Predictions of the average time when most larvae molted to a certain stage under

Figure 19. *Pollicipes polymerus*. Growth of nauplii (mean length ± standard deviation), on several diets, disregarding larval density and naupliar stage. Growth between nauplii eating different foods may be compared. (Larval density does not appear to restrict growth within the density range considered here, as shown by high coefficient of determination values between naupliar stage and time, except in the case of larvae fed *Prorocentrum micans*, which are treated separately.)

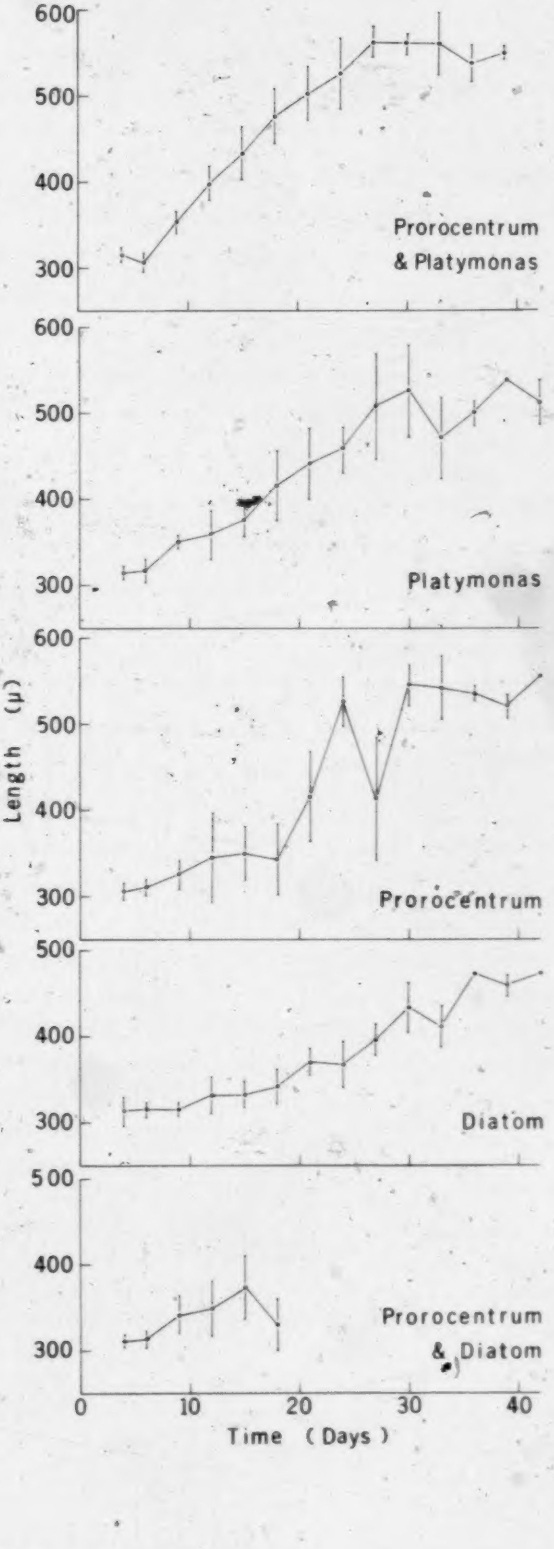

600
500
400
300
Prorocentrum
& Platymonas
600
500
400
300
Platymonas
600
500
400
300
Prorocentrum
500
400
300
Diatom
500
400
300
Prorocentrum
& Diatom
Length (μ)
0
10
20
30
40
Time (Days)

Figure 20. *Pollicipes polymerus*. Growth of nauplii from stage to stage, disregarding larval density in culture. Growth between nauplii eating different foods may be compared. Bars represent percent of the total sample at each stage, the distance on the ordinate representing each stage is 100%. Numbers above the bars are sample sizes. Regression lines and equations are shown.

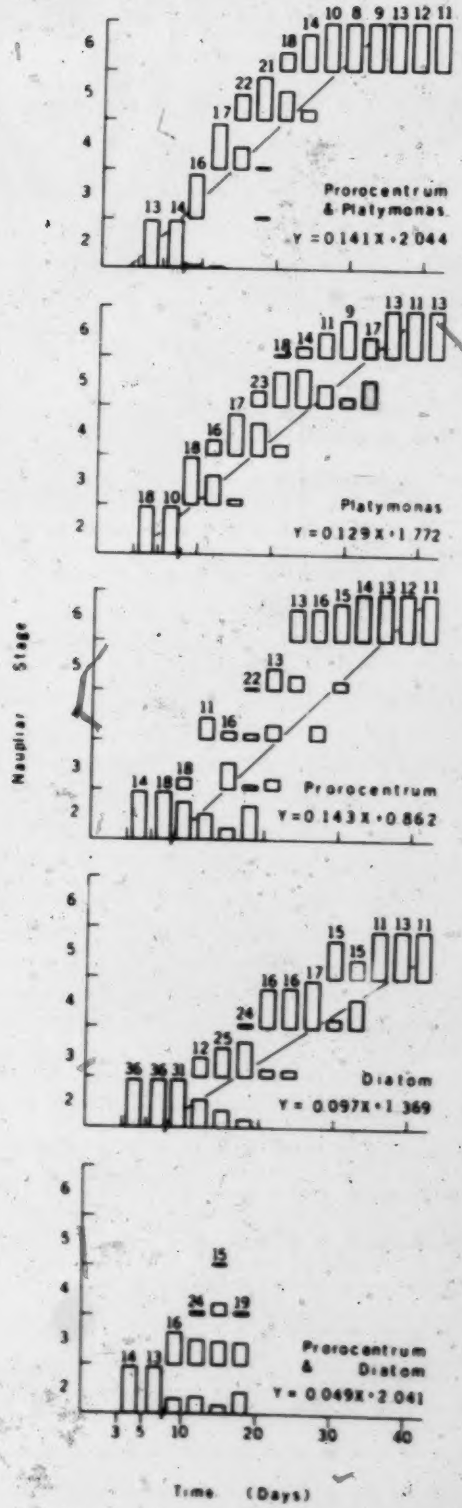

Prorocentrum & Platymonas
Y = 0.141X + 2.044
13 14 16 17 22 21 18 14 10 8 9 13 12 11
Platymonas
Y = 0.129X + 1.772
18 10 18 16 17 23 18 14 11 9 17 13 11 13
Prorocentrum
Y = 0.143X + 0.862
14 18 18 11 16 22 13 13 16 15 14 13 12 11
Diatom
Y = 0.097X + 1.369
36 36 31 12 25 24 16 16 17 15 15 11 13 11
Prorocentrum & Diatom
Y = 0.049X + 2.041
14 13 16 26 15 19
2
3
4
5
6
Naupliar Stage
3
5
10
20
30
40
Time (Days)

Table 14. Pollicipes polymerus. *Statistics describing naupliar growth in culture. NS: naupliar stage; CC: correlation coefficient; CD: coefficient of determination.*

Food	Larval density (larvae/cm^3)	Weighted average NS observed ($\bar{Y}$)	CC between NS and time (r)	CD between NS and time (r^2, in %)	Ranking of Food Using $\bar{Y}$	
					Larval density of *Prorocentrum micans* Considered	Larval density of *Prorocentrum micans* Not Considered
Prorocentrum micans	*	3.29	0.81	65.6	-	3
Prorocentrum micans	0.5	4.45	0.92	84.3	2	-
Prorocentrum micans	1.8	2.40	0.61	37.4	6	-
Platymonas sp.	*	3.96	0.86	74.5	3	2
Diatom	*	2.76	0.90	80.5	4	4
Diatom/*Prorocentrum micans*	*	2.62	0.34	11.8	5	5
Platymonas sp./*Prorocentrum micans*	*	4.58	0.90	80.5	1	1

*Larvae in both densities (0.5 and 1.8 larvae/cm^3) are considered together since only nauplii fed *Prorocentrum micans* had different growth rates when placed in cultures of different larvae-to-food ratios.

Table 15. Pollicipes polymerus. *Predicted average time* (A) *and actual time range* (R) *in days taken to reach a particular naupliar stage after hatching. Only nauplii fed* Prorocentrum micans *had different growth rates when placed in cultures of different larvae-to-food ratios, and therefore are treated separately as well as together here. P.m.: Prorocentrum micans; P.*sp.: *Platymonas* sp.

Stage	Food											
							0.5 larvae/cc		1.8 larvae/cc			
	*P.m./P.*sp.		*P.*sp.		*P.m.*		*P.m.*		*P.m.*		Diatom	
	A	R	A	R	A	R	A	R	A	R	A	R
2	3.2	1-12	5.6	1-6	11.5	1-18	5.2	1-9	15.7	1-18	11.7	1-18
3	10.3	9-18	13.4	9-15	18.4	9-21	12.8	9-18	27.8+	15-21	22.0	12-24
4	17.4	12-18	21.2	12-21	25.4	12-27	20.5	12-21	40.0+	21-27	32.3	21-33
5	24.5	15-24	28.9	18-33	32.4	18-30	28.1	18-30	51.8*	24-	42.6	30-
6	31.6	21-	36.7	21-	39.4	24-	35.7	24-	63.9*		52.9*	

* Extrapolated values

+ Predicted average times do not fall within the range of actual times due to a low correlation coefficient between naupliar stage and time.

culture conditions were made by inverse estimation (Williams, 1959; and Table 15 of present paper). However, larvae fed *Prorocentrum micans* alone in the low larval density culture or *Platymonas* sp. alone, grew almost as fast as those on the combination diet. A few reached Stage 6 the same time or 3 days later than those eating the combination food; and about 32% of the larvae eating *Prorocentrum micans* alone developed to the cypris stage, while only about 3% of those eating the combination food grew to that stage.

In a subsequent experiment, approximately 75,000 Stage 1 nauplii were cultured in 6 4-L beakers with *Prorocentrum micans/Platymonas* sp. Little mortality (15%) to Stage 6 was observed with this method.

The growth rate of barnacles fed the diatom alone was slower than for those fed any other food. Larvae fed the *Prorocentrum micans*/diatom combination began with a faster growth rate, but most larvae died before reaching Stage 4. Possibly some negative interaction of the *Prorocentrum micans*/diatom food affected the larvae fed this combination, since they died by the 18th day after hatching while those larvae fed either food separately lived at least to the 42nd day. This food combination may not have the essential nutrients necessary for larval growth past the critical Stage 3.

If interactions between algal food species do occur, by producing growth stimulators or inhibitors, or by other means, they may be quantified with Interaction Index, I, calculated from the formula:

$I = L_{C1} - [y\,L_{A1} + (1 - y)\,L_{B1}]$ where L_{C1} is the mean larval length of specimens eating the mixed food on Day 1; L_{A1} is the mean length of those eating the first alga on Day 1; L_{B1} is the mean length of larvae eating the second alga on Day 1; y is the fraction of the first alga added to the mixture (calculated using total cell volume; Fig. 21). In determining the food "interactions," it should be kept in mind that the proportion of foods available was taken into account, but the assumption that the larvae will eat the food in this proportion may not always hold.

Effects of Larvae-to-Food Ratio on Larval Development

The amount of variation in growth rates that is explained by growth alone (r^2) in larvae eating *Prorocentrum micans* is low (65.6%). However, when these larvae are divided into the 2 larval density groups, more of the total variation appears to be explained. That is, growth depended on larval density as well as food type and time in these larvae. When the rate of growth of larval length and the rate of transformation from 1 stage to the next were plotted for larvae in the 2 density groups (Figs. 22 and 23), differences were observed. When the *P. micans* concentration was kept constant, an increased number of larvae depressed the larval length and width from 6 to at least 27 days after hatching (Fig. 22). Growth from stage to stage was also slower in high larval density cultures (Fig. 23). Stage 2 lasted much longer (18 days) in the higher larval density culture than in the lower larval density culture (9 days). A decrease in larval size was also observed in both cultures 27 days after hatching, corresponding to a decrease in food concentration by 50%. However, the amount of variation in growth due to time, food type and larval density in the 1.8 larvae/cm^3 culture fed *Prorocentrum* is still low; more factors must be involved.

In cultures fed *Prorocentrum micans/Platymonas* sp., high-density specimens were delayed 6 days at Stage 2 and 9 days at Stage 3 compared to low-density specimens. Larvae in the 2 densities were equally active

Figure 21. *Pollicipes polymerus*. Effect of diet on naupliar growth Filled circles: *Platymonas* sp. plus *Prorocentrum micans*; open circles: *Prorocentrum micans* plus unknown diatom. The Interaction Index expresses the effectiveness of diet in promoting growth (see Interaction Index equation in text).

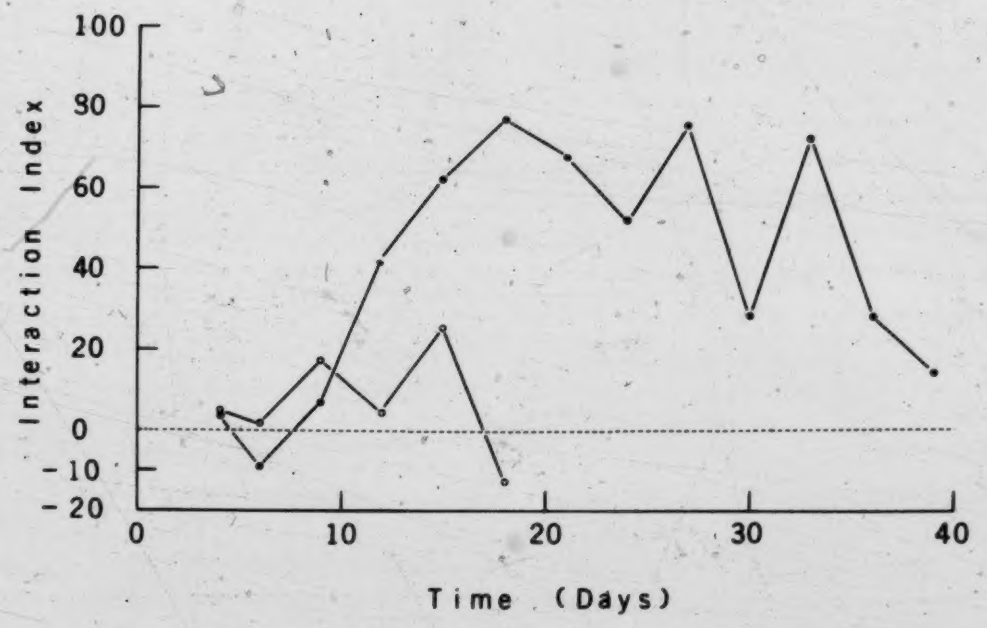

Interaction Index
100
80
60
40
20
0
-10
-20
0
10
20
30
40
Time (Days)

Figure 22. *Pollicipes polymerus*. Growth of naupliar length and width (mean ± standard deviation), disregarding naupliar stage for nauplii eating *Prorocentrum micans* at 2 larval densities in culture. Open squares: length of individual at a density of 1.8 larvae/cm^3; filled squares: width at a density of 1.8 larvae/cm^3; open circles: length at a density of 0.5 larvae/cm^3; filled circles: width at a density of 0.5 larvae/cm^3.

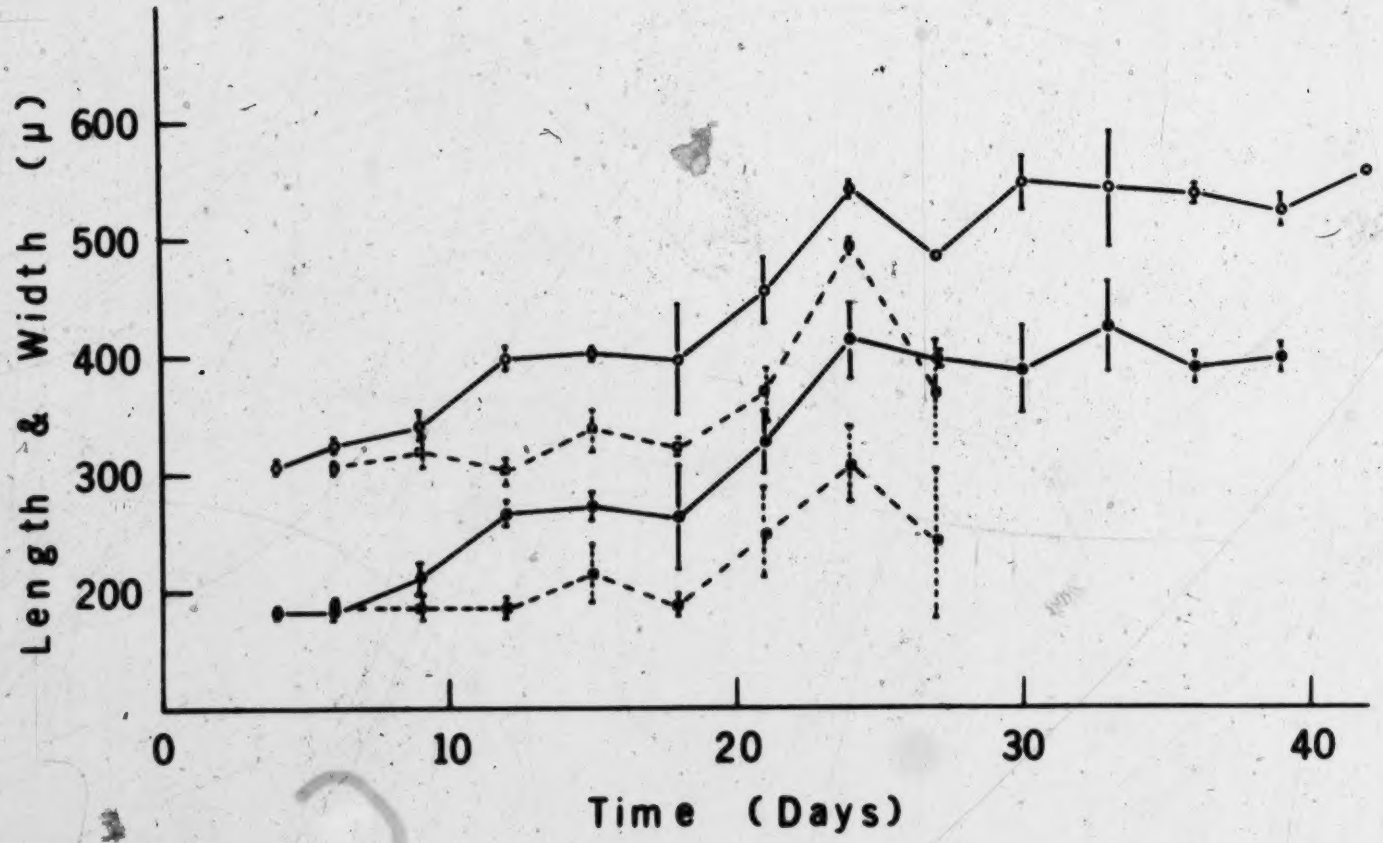
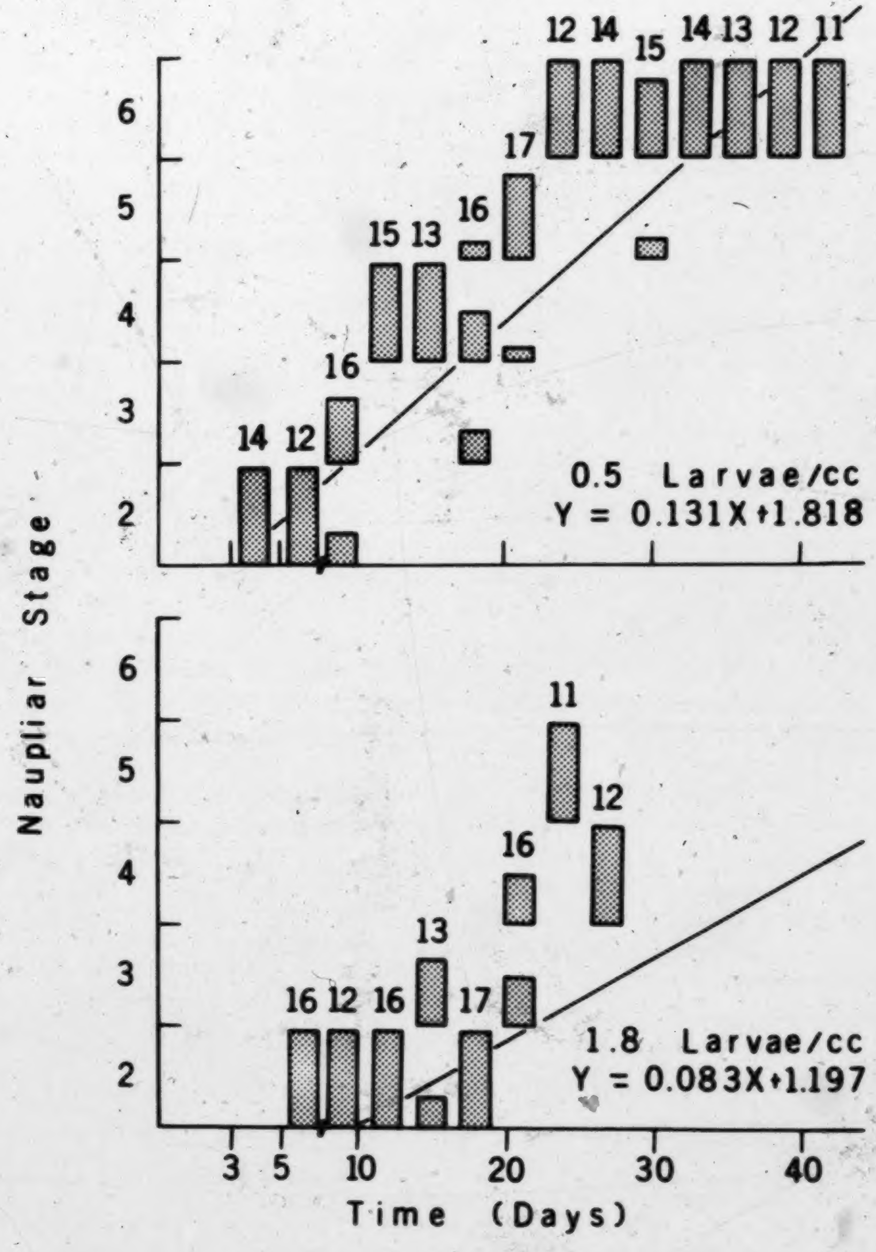

Length & Width (μ)
600
500
400
300
200
0
10
20
30
40
Time (Days)

Figure 23. *Pollicipes polymerus*. Growth of nauplii from stage to stage for larvae eating *Prorocentrum micans* in culture. Growth between nauplii at 2 larval densities may be compared. Bars represent percent of the total sample at each stage, the distance on the ordinate representing each stage is 100%. Numbers above the bars are sample sizes. Regression lines and equations are shown.


Naupliar Stage
6
5
4
3
2
14
12
16
15
13
16
17
12
14
15
14
13
12
11
0.5 Larvae/cc
Y = 0.131X+1.818
6
5
4
3
2
11
12
16
13
16
12
16
17
1.8 Larvae/cc
Y = 0.083X+1.197
3
5
10
20
30
40
Time (Days)

until 39 days after hatching. At this point the setae of larvae in the low larval density culture became clogged with *Platymonas* sp. While lateral eye pigmentation developed in the low-density larvae first (27 days after hatching) compared to the high-density specimens (30 days after hatching), cyprids developed only in the latter group.

Although no size or growth rate differences were noted for specimens in the 2 larval densities grown in *Platymonas* sp. alone, more vigorous feeding and swimming activity was observed in the culture with lower larval density from the 24th to the 39th day after hatching. On the 30th day after hatching, the *Platymonas* sp. concentration was increased by 50% in both cultures to determine the effect of increased food availability. On the 39th day after hatching, larvae in the lower density larval culture became entrapped in *Platymonas* sp. and its mucus. By the 42nd day after hatching, larvae in both cultures experienced this problem and most died soon after.

Other density effects were observed: in cultures fed *Prorocentrum micans*/diatom, a few low-density animals grew to Stage 5, whereas no high-density larvae lived past Stage 4. When larvae were fed the diatom alone, decreased activity and increased mortality rates were observed first in the low larval density culture (27th day after hatching) followed by the high larval density group (39th day after hatching). On the 30th day after hatching, the larvae from both cultures became entangled in algal mucous nets.

Discussion

Embryonic Growth in vitro

From early development to second cleavage, the data from Nussbaum

(1890, culture temperature not indicated) agree approximately with the rate of development of *Pollicipes polymerus* at 13° to 15°C (Lewis, 1975b; see Table 16 of present paper). Development from gastrulation to appendage bud formation took 1 day longer under the conditions of Nussbaum's cultures. The naupliar eye developed more slowly but hatching occurred 5 days earlier. Hilgard's cultures (1960, 13°C) of *P. polymerus* grew more slowly at all phases, taking 5 days longer to develop to hatching. Hilgard's data agree more closely with those given for *P. spinosus* by Batham (1946) than for *P. polymerus* (Lewis, 1975b). However, it is reasonable to expect that the yolky embryos of *P. spinosus* would take longer to complete embryonic development than the smaller, less yolky eggs of *P. polymerus* (Anderson, 1965, 1973).

It has been shown that embryonic developmental rate is a function of temperature in several barnacle species (Crisp, 1959b; Patel and Crisp, 1960b; Crisp and Costlow, 1963). Thus, it is possible that the developmental rate differences between the current (Lewis, 1975b) and the 2 previous studies (Nussbaum, 1890; Hilgard, 1960) are primarily due to variations in culture temperature. On the other hand, the 5 day difference in developmental times for *P. polymerus* is probably too great to be explained by a single factor, since a 2 °C difference in temperature (14 minus 12°C) only made a difference of 2 days in developmental time for *Balanus balanoides* at a late stage (Crisp, 1959). Other factors (photoperiod, lamella size, amount of aeration, and addition of drugs) also affected developmental time (Table 13). Of these factors, aeration appears to be one of the most consistent since it shortened developmental time in 76% of the cases observed.

Table 16. Pollicipes spp. *Time when event occurs for majority of embryos observed in culture. Developmental timetable (after Lewis, 1975b). Data from other authors is compared.*

Stage	(13-15°C) Lewis, 1975b	(13°C) Hilgard, 1960	(?°C) Nussbaum, 1890	(14-15°C) *P. spinosus* Batham, 1945
Unfertilized eggs (sperm added)	0			
Eggs round up, fertilization membrane	0.3 h			
Fertilization membrane becomes sticky	0.6 h			
First peristaltic constriction rings	1.3 h			
First polar body, 2 constriction rings	2.0 h			
3-5 peristaltic constriction rings, eggs elongated	2.2 h		first seen (x)	
Egg membrane begins lifting	2.2 h			
1-2 peristaltic constriction rings	4.8 h			
1-2 peristaltic constriction rings, second polar body	5.6 h		x + 6 h	
Egg membrane lifted	8.3 h			first seen as single cell (x)
First cleavage	29.0 h	first seen (x)	x + 26 h	x + 1 day
Second cleavage	30.0 h		x + 1 day	
Third cleavage	37.5 h			
Fourth cleavage	55.2 h			x + 3 days
Fifth cleavage (31 cells)	60.0 h			
Sixth cleavage	65.0 h			
Gastrulation by epiboly	3.1 days		x + 4 days	x + 6 days
Division of internal yolky cell	4.4 days		x + 4 days	x + 4 days
Formation of mesoblast cells	5.1 days			x + 7 days
Segmentation	6.8 days		x + 7-8 days	x + 8 days
Naupliar appendage buds	9.0 days	x + 10 days	x + 10 days	x + 9 days
Origin of labrum and gut	11.3 days			x + 13-14 days
Appendages with setae, origin of coelom	12.6 days			x + 14 days
Naupliar eye, pronephros, horn gland	13.3 days	x + 16 days	x + 16 days	x + 14-17 days
Limb movement within egg membrane, body transparent except for gut	16.7 days			x + 19-24 days
Hatching	25.4 days	x + 29-31 days	x + 20 days	x + 30-32 days

Crisp (1959b) found that the youngest embryos are most affected by culture conditions. If it is assumed here that the addition of drugs (10.9 days, no drugs minus 9.6 days, full-strength penicillin-streptomycin-chloromycetin = 1.3 days) plus the breaking of lamellae into 2 pieces (0.2 days) increased the developmental rate of *P. polymerus* by 1.5 days from labrum formation to hatching, it is likely that the developmental rate of earlier stages was increased by 2 to 3 times that amount (3 to 5 days). The 5-day difference between developmental time to hatching in this (Lewis, 1975b) and the other 2 studies (Nussbaum, 1890; Hilgard, 1960) can be accounted for by assuming a 1-day difference due to temperature plus an average of 4 days due to addition of drugs and cutting the lamellae into 2 pieces. It is also likely that the recording of time until hatching (duration of the eye-stage) varies with the definition of when hatching commences used by various investigators. The mean time from fertilization to hatching observed by Lewis (1975b; 25.4 days) lies halfway between the times found by Nussbaum and by Hilgard and the range includes their points.

The rate of aerobic respiration of the embryo mass depends on the size and shape of lamellae and the amount of aeration (Crisp, 1959b) and on temperature. A higher density of entangling blue-green algae and diatoms developed in embryo cultures grown in natural illumination than in those grown in darkness. Addition of drugs apparently reduced bacterial and fungal contamination, which would have limited water circulation through the embryo mass and have used up some of the available oxygen. This reduction of contamination slightly increased the rate of embryonic development.

It is possible that the drug dosage was too high and that the metabolism of some embryos was changed, thus altering slightly the pattern of development and causing some abnormalities.

Optimum culture conditions would probably consist of: aeration, drugs at slightly lower dosage than the half-strength used, darkness, and lamellae cut into pieces.

Naupliar Growth in vitro

From the preliminary experiment, it was observed that naupliar growth and development depend primarily on the species of algal food available. Bivalve larvae can be killed by high concentrations of algal cells and their filtrates (Loosanoff *et al.*, 1953), i.e., both mechanical interference of food cells with larval swimming and feeding mechanisms and toxic metabolites secreted by the algae may be lethal. This appears to be true with *P. polymerus* nauplii (Table 10).

Wisely (1960) showed that cultures with low larval density had lower mortality rates and grew faster than those in crowded cultures. In the present experiments, only *Prorocentrum micans*-fed nauplii were affected significantly by larva to food ratios (density). Apparently, in all cultures except the high larval density culture fed *P. micans*, a sufficient algal concentration was available to sustain a fast growth rate.

A nearly optimum feeding schedule for *P. polymerus* nauplii in culture might be: 500 cells/mm^3 of *Platymonas* sp. for the first 9 to 10 days after hatching, and thereafter 5 to 10 cells/mm^3 of *Prorocentrum micans* added to the *Platymonas* sp. until the Stage 6 nauplius is reached. *Platymonas* sp., a small flagellate, is probably ingested more easily, particularly by young nauplii, than *Prorocentrum micans*, a large dinoflagellate. However, the addition of *Prorocentrum micans* to *Platymonas* sp. appears to encourage larval growth in the later stages (Fig. 19) and a positive interaction is implied from the higher growth rate observed when the 2 algae are eaten together (Fig. 21). It is likely that each alga lacks a different nutrient necessary for optimal growth of *Pollicipes polymerus* larvae, and when both algae are available as food, neither nutrient is lacking.

The negative interaction observed for this combination of algae during the first few days of naupliar growth (Fig. 21) may be due to the larvae spending energy attempting to eat the *Prorocentrum micans* which is too large to ingest at this time. Larvae fed *Platymonas* sp. alone did not develop into viable cyprids and most did not even develop compound-eye pigmentation in Stage 6. The setae of Stage 6 larvae (39th day after hatching) eating *Platymonas* sp. alone or in combination with *Prorocentrum micans* became entangled with the *Platymonas* sp. This is probably due to the great number of plumose and feathery setae which develop at this stage (Lewis, 1975b) and to the long duration of Stage 6 before metamorphosis to the cypris larva. Only 3% of the larvae eating *Prorocentrum micans/Platymonas* sp. metamorphosed to cypris. Decreasing the *Platymonas* sp. concentration when larvae reach Stage 6 might eliminate this problem. Even when fed *Prorocentrum micans/Platymonas* sp. throughout naupliar life, 85% of the larvae reached Stage 6.

The unknown pennate diatom must have provided most of the nutrients necessary for larval growth to Stage 5 (Fig. 20). Although the diatoms were observed in the gut, some factor critical to molting may have been lacking in the diet, molting was inhibited, or some other growth factor may have been involved. In any case, at the time when addition of

Prorocentrum micans to *Platymonas* sp. enhanced larval growth rates, growth declined in the barnacles fed the *Prorocentrum micans*/diatom until all the larvae died. Although the early death of larvae fed the *P. micans*/diatom diet occurred in 2 separate cultures, it may be attributable to uncontrolled variables other than diet.

DEVELOPMENT: FERTILIZATION THROUGH SETTLEMENT[6]

Introduction

Pollicipes polymerus (Sowerby, 1833) is an exclusively littoral species (Pilsbry, 1907; Barnes and Reese, 1960) found in the upper two-thirds of the intertidal zone, commonly associated with *Mytilus californianus* and *Pisaster ochraceus* along the open California coast (Ricketts and Calvin, 1968) in areas with strong wave action (Cornwall, 1969). *P. polymerus* appears to occur only in dense clusters and is rarely solitary (Shelford, 1930; Towler, 1930; Barnes and Reese, 1960).

Although this species is one of the most common intertidal invertebrates of the unprotected, rocky shore of the West Coast of North America, little of its embryological development and nothing of its larval development or of its settling behavior has thus far been known. Attempts to describe naupliar development to the cypris stage (Hildreth, 1950, Snodgrass, 1967) have been unsuccessful. It is important, then, to learn the details of development in this animal so that its embryos may be cultured for future experiments and its larvae easily recognized in plankton samples.

Nussbaum (1890) accurately described first cleavage, polar body formation and gastrulation in *Pollicipes polymerus*, but gave no detailed account or timetable of developmental events. The naupliar outline and internal organs observed by Nussbaum (1890) of a Stage 1 nauplius appear to be correct, but the setation is inaccurate. Groom's (1894) notation for staging *Balanus perforatus* and *Lepas anatifera* embryos was also used in reference to *P. polymerus* embryos by Barnes and Barnes (1959a), although the emphasis was not on a description of the developmental events. A comprehensive review of cirripede embryonic development is given by Anderson (1973).

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[6] In press in *Marine Biology*

This chapter describes the embryonic and larval development and settlement of *Pollicipes polymerus* and compares its naupliar stages with those of other pedunculate species.

Materials and Methods

Fertilization Events

Pollicipes polymerus were collected with care to insure healthy adults when *in vitro* fertilization was attempted. They were most easily removed intact when attached to *Mytilus californianus.* Many barnacles were opened until one was found with sperm already deposited in the mantle cavity. These were the only sperm which successfully fertilized the eggs (see also Walley *et al.*, 1971). Sperm, eggs from the ovary or oviduct ready to ovulate, and oviducal gland fluid were mixed and kept at ambient sea water temperature. The most successful observations of fertilization events were accomplished with individual eggs.

Culture of Embryos and Larvae

Egg masses (lamellae) with newly-fertilized eggs were taken from adult mantle cavities. Fertilized eggs (*in vitro* as well as *in vivo*) were cultured in grossly-filtered, Millipore-filtered or autoclaved sea water in small finger bowls at ambient sea water temperature (summer at Friday Harbor, Washington: 12° to 15°C; Bodega Bay, California: 13° to 16°C). Streptomycin and penicillin (Tighe-Ford *et al.*, 1970) and 1 mg/L chloromycetin (Barnes and Barnes, 1959) kept bacterial and fungal growth low. Fresh, aerated sea water was added daily.

Nauplii were collected upon hatching and raised *in vitro* through all 6 stages (Lewis, 1975a). Larval stages were described and measured using a Wild M-20 compound microscope, calibrated ocular micrometer and drawing tube. The nauplii were transferred to 85% lactic acid for easy manipulation (Dudley, 1957), stained with alcoholic black E (Perkins, 1956) in lactic acid for 2 to 12 h, depending on the stage, and dissected with fine tungsten needles (0.02 cm diameter, eroded to a point using molten sodium nitrite). An identified series of larval stages is deposited with the National Museum of Natural History in Washington, D.C., U.S.A. (NMNH 150124).

Description of Larvae

Nauplii from each of the stages of several broods were measured. Total length was measured from the extreme anterior part of the body to the tip of the caudal spine or abdominal process, whichever was longer. Width was measured at the widest portion of the carapace and length of the carapace (distinct from the body in Stages 4 through 6) was measured from the extreme anterior part of the body to the most posterior part of the carapace.

Settlement of Cyprids

The sea water was no longer changed in laboratory cultures once cyprids appeared. The cyprids were presented with various substrates to determine some factors responsible for stimulating settlement: 1) plain and etched glass slides (set in flowing sea water for 48 h to develop primary films); 2) slides plus whole adult *Pollicipes polymerus* extract; 3) whole adult *P. polymerus* extract (in glass beakers); 4) healthy adult *P. polymerus*; 5) epidermis from the adult *P. polymerus*; 6) mudstone from Duxbury Reef, California, U.S.A.

Results

Embryogenesis

From the culture of embryos, a developmental timetable was constructed (Table 17). The egg of *Pollicipes polymerus* was yolky and golden yellow-orange in color. The fertilized egg averaged about 100 μ in diameter. Before fertilization, it was irregular in shape; after fertilization, it became spherical and then elongated to a shape resembling a hen's egg.

The fertilization membrane lifted from the egg, became sticky, and appeared to be responsible for the adhesion of eggs to each other (see also Walley *et al.*, 1971). The first polar body formed at the egg's animal (blunt) pole. A description and preliminary analysis of the peristaltic constriction phenomenon has been given (Lewis *et al.*, 1973). This active movement, coupled with ooplasmic segregation of yolk platelets to the vegetal pole, suggests a reorganization of other materials as well as the yolk at this time. Globules of lipid yolk were identified moving vegetally. The tough, ovoid egg membrane was first observed at points of the constriction rings. It provided protection to the embryo and rigidity to the egg mass. The second polar body formed within the egg membrane. After fertilization, there was a shrinkage in embryo volume (Table 17, first constriction stage) followed by a gradual increase in volume. This has been noted in other barnacle species (Groom, 1894; Crisp, 1954).

As a result of the preponderance of yolk, cleavage was modified

Table 17. Pollicipes polymerus. *Developmental timetable with embryo sizes (measured inside egg membrane). n: no. of specimens observed.*

Stage	Mean embryo size (μ)			Time when event occurs for majority of embryos observed in culture (13-15°C)		
	n	Greatest Width	Length	n	Mean	Range
Unfertilized eggs (sperm added)	10	80	105	10	0	
Eggs round up, fertilization membrane	10	100	100	6	0.3 h	0.2-0.5 h
Fertilization membrane becomes sticky	10	100	100	1	0.6 h	-
First peristaltic constriction rings	9	60	140	7	1.5 h	0.6-3.5 h
First polar body, 2 constriction rings	6	60	140	7	2.0 h	0.2-2.2 h
3-5 peristaltic constriction rings, eggs elongated	9	85	140	4	2.2 h	2.0-4.2 h
Egg membrane begins lifting	9	85	140	3	2.2 h	2.0-4.2 h
1-2 peristaltic constriction rings	9	85	140	3	4.8 h	4.0-6.0 h
1-2 peristaltic constriction rings, second polar body	10	90	140	3	5.6 h	4.0-6.0 h
Egg membrane lifted	10	93	140	3	8.3 h	6.7-9.0 h
First cleavage	10	93	140	6	29.0 h	22.0-50.0 h
Second cleavage	10	95	140	5	30.0 h	24.0-39.5 h
Third cleavage	10	100	145	6	37.5 h	29.7-46.5 h
Fourth cleavage	10	100	145	6	55.2 h	46.2-67.2 h
Fifth cleavage (31 cells)	10	90	130	2	60.0 h	52.0-68.0 h
Sixth cleavage	5	90	135	2	65.0 h	59.8-71.2 h
Gastrulation by epiboly	10	80	100	7	3.1 days	2.1-4.3 days
Division of internal yolky cell	10	80	110	11	4.4 days	2.5-8.6 days
Formation of mesoblast cells	7	90	130	2	5.1 days	4.2-6.0 days
Segmentation	20	80	120	13	6.8 days	4.1-11.1 days
Naupliar appendage buds	10	100	160	13	9.0 days	5.2-15.3 days
Origin of labrum and gut	10	115	185	11	11.3 days	9.7-16.3 days
Appendates with setae, origin of coelom	10	115	185	5	12.6 days	11.1-15.1 days
Naupliar eye, pronephros, horn gland	10	130	200	10	13.3 days	11.7-16.1 days
Limb movement within egg membrane, body transparent except for gut	3	135	220	5	16.7 days	15.3-18.1 days
Hatching	10	130	250	8	25.4 days	20.3-30.1 days

spiralian and unequal. During the first cleavage, which was equatorial, the blastomeres rotated within the egg membrane, producing an anterior AB micromere and a posterior CD macromere. Although the lineage of individual cells was not followed throughout development, it appeared that the rest of development was similar to that described for other hermaphroditic barnacles with planktotrophic larvae, e.g. *Lepas anatifera*, *Balanus perforatus* and *Chthamalus stellatus* (Groom, 1894), *B. balanus*, *B. balanoides* (Barnes, 1965) and *Tetraclita rosea* (Anderson, 1969). However, it differed from barnacles with lecithotrophic development, e.g. *Pollicipes spinosus* (Batham, 1946) and *Ibla quadrivalvis* (Anderson, 1965; see also Anderson, 1973). Embryonic development is shown in Figures 24 and 25.

A fairly reliable method of estimating the developing embryonic stages uses lamella color and texture. Thus, lamellae bearing eggs just fertilized were pale orange, flimsy as determined by manipulation, and rounded. In those with young embryos (formation of blastoderm to formation of naupliar segments) lamellae were light orange, firm, brittle and flattened. In advanced embryos (marking out of naupliar appendages to origin of body cavity) the lamellae were bright orange, firm, brittle and flattened. In the pre-hatching stage with naupliar eye, the lamellae were dark orange, brown or dark pink, flattened, crumbling or fragmented.

Embryos cultured singly developed slightly faster than those in lamellae, and embryos on the periphery of the lamellae were slightly ahead of those nearer the center. The mean and range of time to each developmental stage from fertilization are given in the developmental timetable (Table 17).

Figure 24. *Pollicipes polymerus*. Embryonic development, all photographs are from living specimens; A and B using Nomarski optics, C to I using phase-contrast optics.

A. Just fertilized egg, showing the fertilization membrane (f) starting to elevate; note homogeneous yolk globules. X 300.

B. Egg with the first polar body formed (p1); note 2 shallow peristaltic constriction rings. X 300.

C. Fertilized egg with peristaltic constriction rings increasing in intensity and number (5); egg membrane (e) beginning to lift; note ooplasmic segregation of yolk droplets from the animal pole (a) to the vegetal pole (v); fertilization membrane is removed. X 430.

D. Egg with the second polar body (p2) formed; ooplasmic segregation almost completed. X 400.

E. 2-cell stage, after rotation of the cleavage plane; note the lipid droplets in the CD macromere. X 300.

F. 4-cell stage with nuclei at metaphase; note the D macromere with the majority of yolk. X 300.

G. 6- to 8-cell stage; note the 1D macromere with the majority of the yolk. X 260.

H. 28- to 33-cell stage; micromeres moving to cover the macromere by epiboly. X 400.

I. Gastrula, showing the blastoderm around the central yolky cell and the blastopore is closed. X 520.

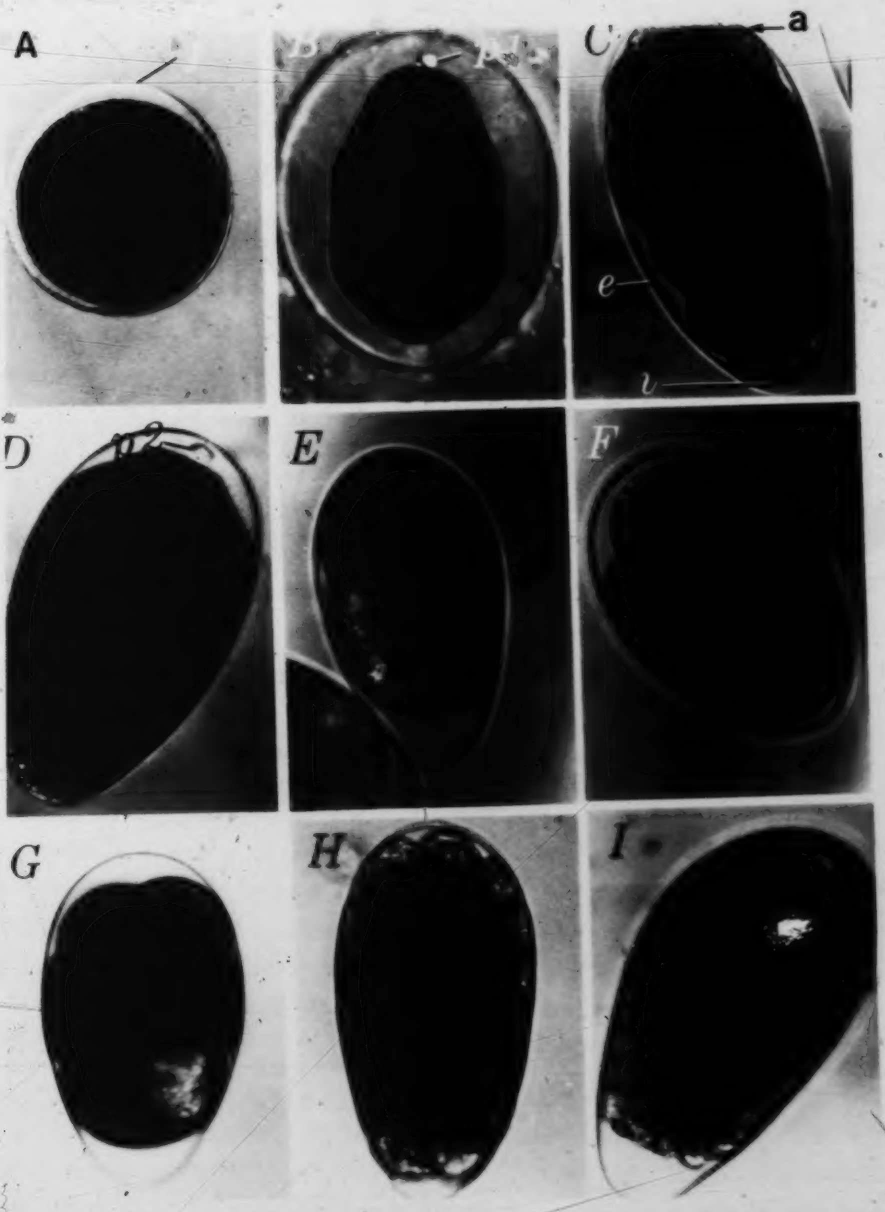

A
B
p.b.
C
a
e
i
D
E
F
G
H
I

Figure 25. *Pollicipes polymerus*. Embryonic development, all photographs are from living specimens; phase-contrast optics.

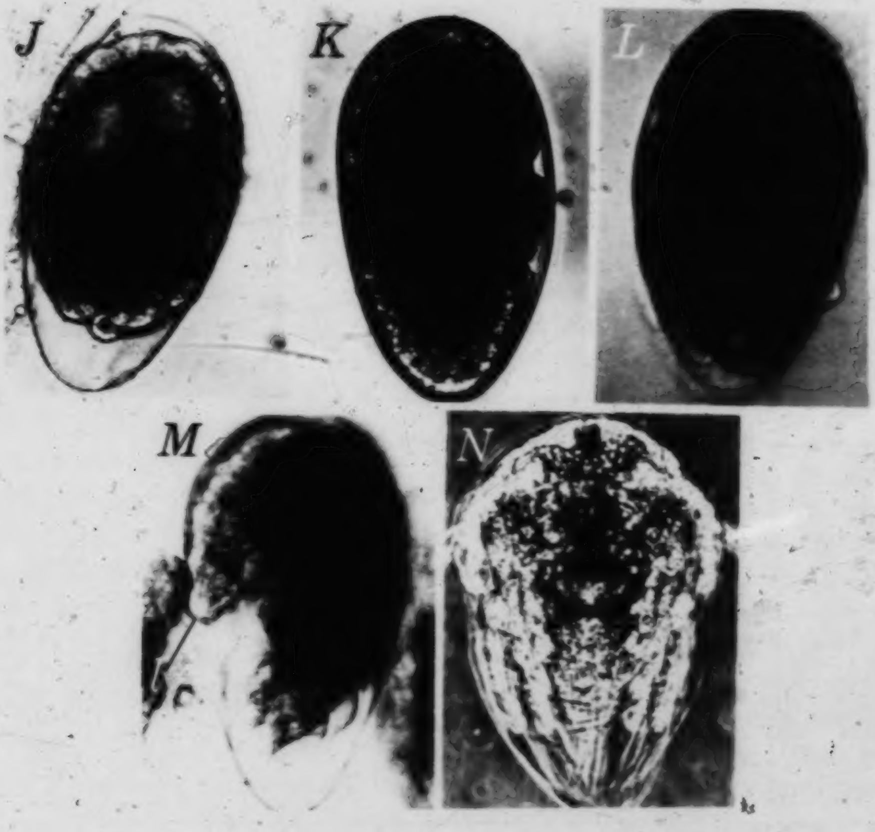
J. Embryo with the yolky cells dividing. X 420.

K. Embryo forming ventral furrows (segmentation); mesoderm is differentiating; lateral view. X 470.

L. Embryo forming 3 paired naupliar appendages (antennules, antennae and mandibles) and post-naupliar region; lateral view. X 340.

M. Embryo with labrum (l) developing, setae appearing on the appendages and yolk becoming restricted to the gut; lateral view. X 300.

N. Hatching nauplius, with a median eye. X 240.


J
K
L
M
N

Hatching and Early Nauplius Stages

Hatching occurred partly as a result of mechanical abrasion with the egg membrane by the actively moving nauplius. Normally, hatching occurred within the mantle cavity or, upon expulsion, in water currents from the adult. When some adults were kept out of water for approximately 12 h to simulate low tide and then reimmersed in dishes with swimming *Artemia salina* for food, they extruded clouds of hatching nauplii. The streams were propelled at least 3 cm from the adult. However, when the lamellae were removed prior to natural hatching and expulsion and cultured *in vitro*, hatching occurred only in part of the population and sporadically over a period of days.

After hatching, the nauplii remained in the first stage from a few minutes up to several hours before molting to Stage 2. First stage nauplii apparently did not feed, since they molted almost immediately to the second stage. At the second naupliar stage, the digestive tract was functional; peristaltic contractions were observed to move food in the gut. Sometimes contractions appeared only in the hindgut. This observation, in conjunction with the fact that starved Stage 2 nauplii died within a few days after hatching without molting, indicates that feeding was obligatory beginning with the second naupliar stage.

Description of Larval Stages

Size ranges. Estimates of the means and standard deviations for size in the larval population are given for each larval stage of *Pollicipes polymerus* reared in the laboratory (Table 18). The ranges of length and width overlap for Stages 2 through 6 and carapace length for Stages 4 through 6.

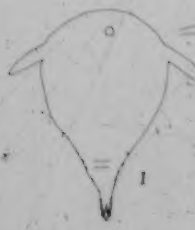
Table 18. Pollicipes polymerus. *Measurements of larval stages. Estimates of the mean and standard deviation for the larval population. S.D.: Standard deviation.*

Stage	n	Length (μ)			Width (μ)			Carapace length (μ)		
		Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
1	8	207	12	182-228	114	24	94-139			
2	232	315	10	287-347	185	8	165-208			
3	144	350	14	307-383	222	10	189-251			
4	120	392	18	346-461	270	14	238-316	271	14	238-307
5	108	465	23	412-507	326	14	288-372	326	19	275-358
6	97	542	30	472-614	390	22	319-483	383	22	332-437
Cyprid	1	425			232					

Compound eyes and carapace. The carapace is nearly as broad as it is wide in Stages 4, 5 and 6 (Table 18) and does not bear posterior carapace spines or any other ornamentation (Fig. 26). The frontolateral horns point posteriorly at approximately a 45° angle from the body in Stage 1. In Stage 2, the horns are almost perpendicular to the body, and from Stage 3 through 6 they are perpendicular or point slightly cephalad. From 6 to 15 days after the sixth naupliar stage is reached, depending upon culture conditions, the 2 lateral compound eyes begin pigmentation.

Labrum. The labrum is unilobed. In Stage 1 nauplii there is a single tooth on each posterolateral margin and a median bilobed tooth (Fig. 27). A few fine frontal hairs, a dorsal row of fine hairs, and minute ventral teeth are present. During the molt between first and second naupliar stages, the lateral teeth shorten and the bilobed teeth grow longer and sometimes are trilobed. The lobed teeth move laterally. Short dorsolateral spines may also be present. The labra of Stage 3 nauplii no longer have lobed teeth. The bilobed teeth appear to become 2 separate teeth and a variable number of small lateral teeth remain. Small single teeth are usually added to the 2 long teeth in Stages 4 and 5. In Stage 6 nauplii, 1 or 2 small teeth lateral to the 2 long teeth and 3 to 4 short dorsolateral teeth are observed on each side. The frontal hairs grow in length and thickness with each increase in stage. Naupliar Stages 2 and 3 have 2 lateral rows of fine dorsal hairs on each side; the median rows are denser. By Stage 4, the rows of hairs have thickened and are equally dense. The inner rows of hairs thicken more in Stage 5 and by Stage 6 the "hairs" are so thick they could be considered long spines. The few small, ventral teeth observed in

Figure 26. *Pollicipes polymerus*. Outline drawings (ventral view) of the 6 naupliar stages.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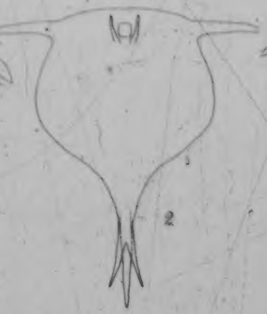
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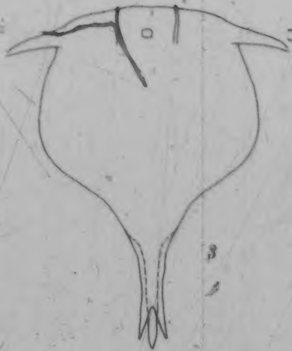
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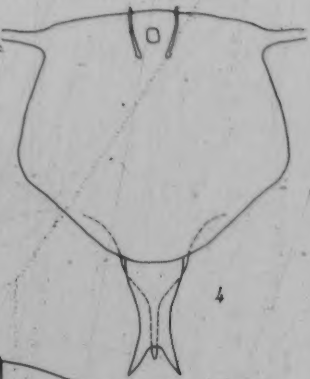
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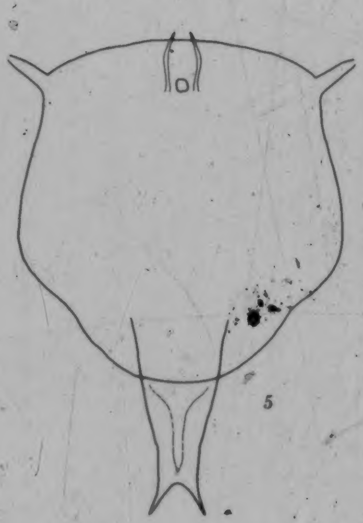
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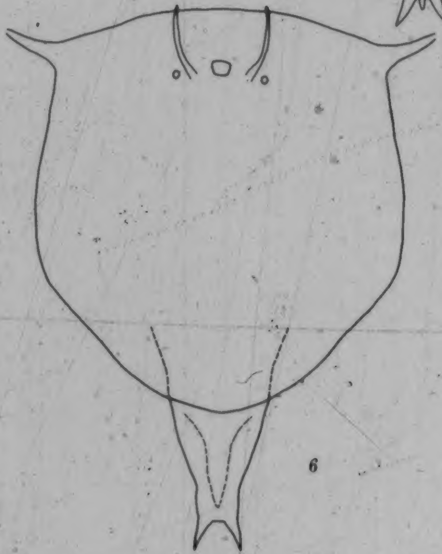
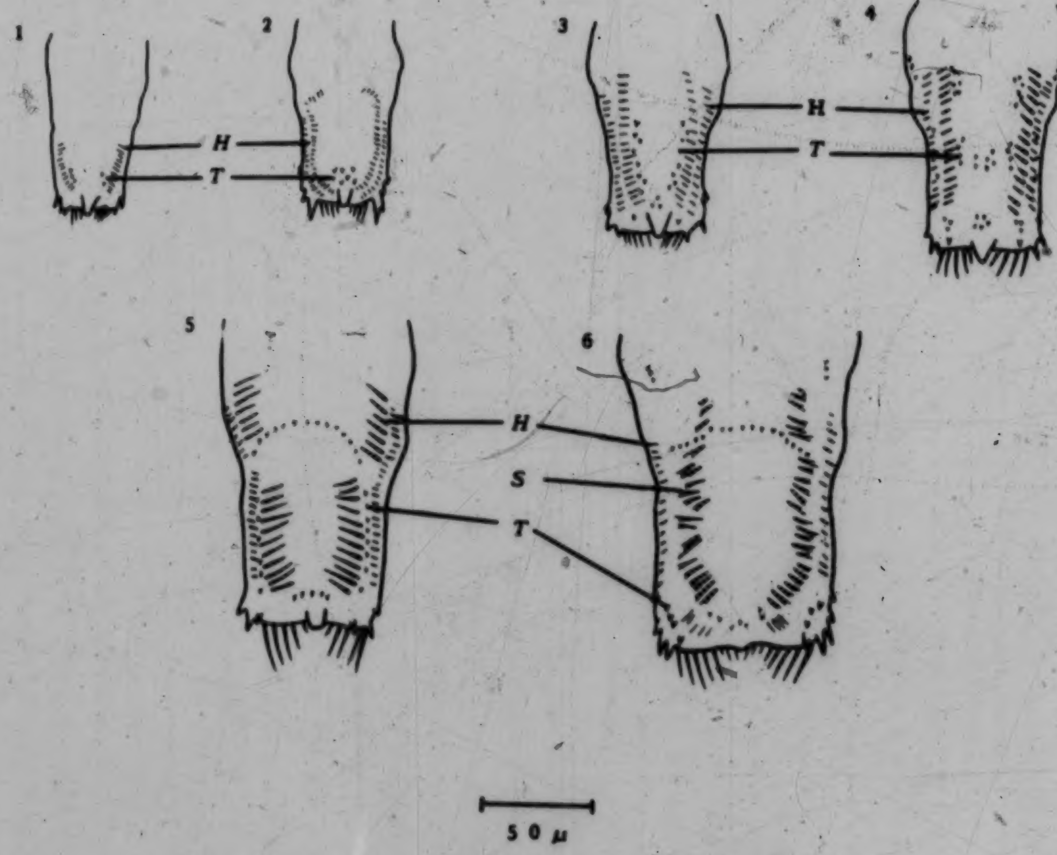

6

50 μ

Figure 27. *Pollicipes polymerus*. Labra of the 6 naupliar stages. The labra bear hairs (H) and spines (S) on the-dorsal surface and teeth (T) on the ventral surface.


H
T
S
50 µ

Stage 2 nauplii also grow in size and distribution with each increase in stage.

Abdominal process and caudal spine. In Stage 1, 2 and 3 nauplii, the caudal spine is longer than the abdominal process, but in Stage 4 nauplii they appear to be subequal, and by Stages 5 and 6 the abdominal process exceeds the length of the caudal spine (Fig. 28). This is known to occur in only 2 other barnacle species (*Chthamalus aestuarii* and *Balanus pallidus stutsburi*) (Sandison, 1967). Although the caudal spine is naked in Stage 1 nauplii, it acquires spinules in the Stage 2 nauplii, and the spinules grow in number and size in later stages. The posterior tips of the process appear to be jointed from Stages 2 through 6 (see lateral view, Fig. 28). The distribution and appearance of abdominal process spines is the same in *Pollicipes polymerus* as in balanoids (Moyse, 1961) except for the absence of the Median Series 2 spine in Stage 4 *P. polymerus* nauplii (Fig. 28). Tiny spinules appear on the abdominal process in Stage 1 nauplii, and grow with increasing stages. Although many were randomly distributed, some patterns are apparent (Table 19).

Appendage setation. The antennules, antennae and mandibles of *Pollicipes polymerus* nauplii, Stages 1 through 6, are illustrated in Figures 29 and 30, and the Newman (1965) type of setation formula using additional setal types suggested by Sandison (1967) is given in Table 20.

Setules found on many setae of early-stage nauplii are very fine, and are seen clearly only when stained. Spatulate setae with fine, feathery setules occur on the antennary endopodite and a single hispid seta is found on the antennary endopodite segment adjacent to the

Figure 28. *Pollicipes polymerus*. Ventral and lateral views of caudal spines (CS) and abdominal processes (AP) of the 6 naupliar stages. Maxillule is seen in lateral view only. Numbers refer to the primary spine series.

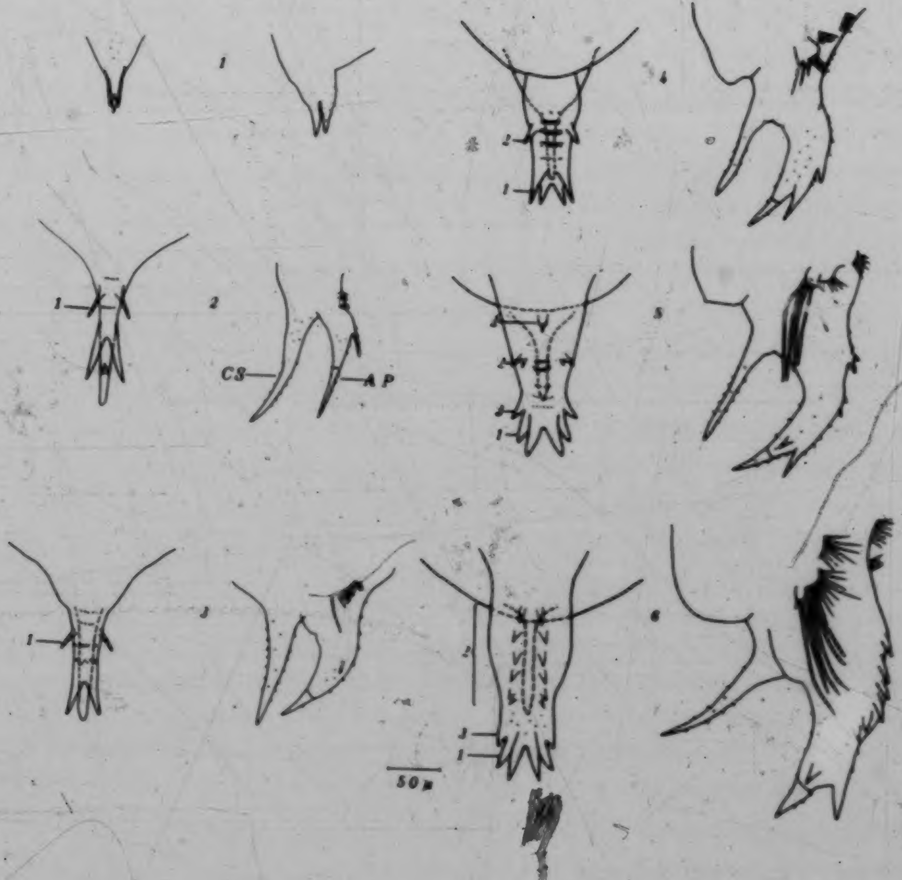

CS
AP
50 µ

Table 19. Pollicipes polymerus. *Distribution of abdominal process spinules.*

Stage	Distribution of rows	Number of rows
1	Random	—
2	Anterior to Series 1 spines	1
2	Posterior to Series 1 spines	1
3	Anterior to Series 1 spines	1
3	Even with Series 1 spines	1
3	Posterior to Series 1 spines	2
4	Anterior to Series 2 spines	2
4	Even with Series 2 spines	1
4	Posterior to Series 2 spines	2
5	Even with Series 2 spines	2
5	Posterior to Series 2 spines	2-3
6	Random	—

Figure 29. *Pollicipes polymerus*. Antennules (left) and mandibles (right) of the 6 naupliar stages.

158

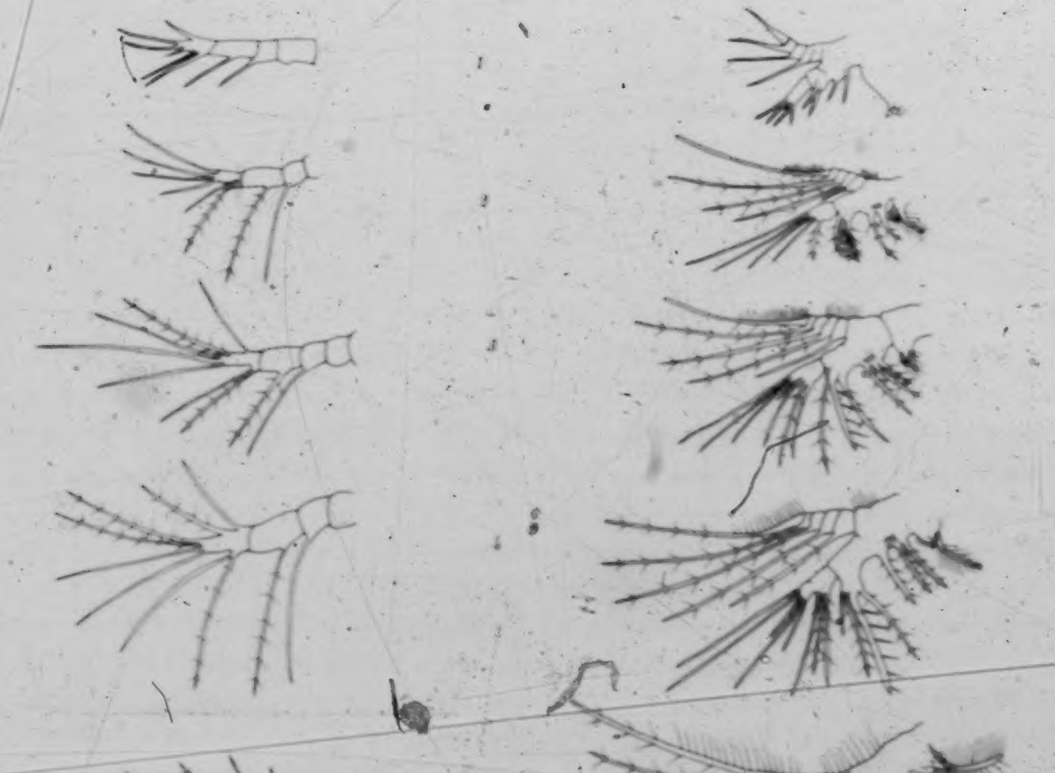


Figure 30. *Pollicipes polymerus*. Antennae of the 6 naupliar stages.

160

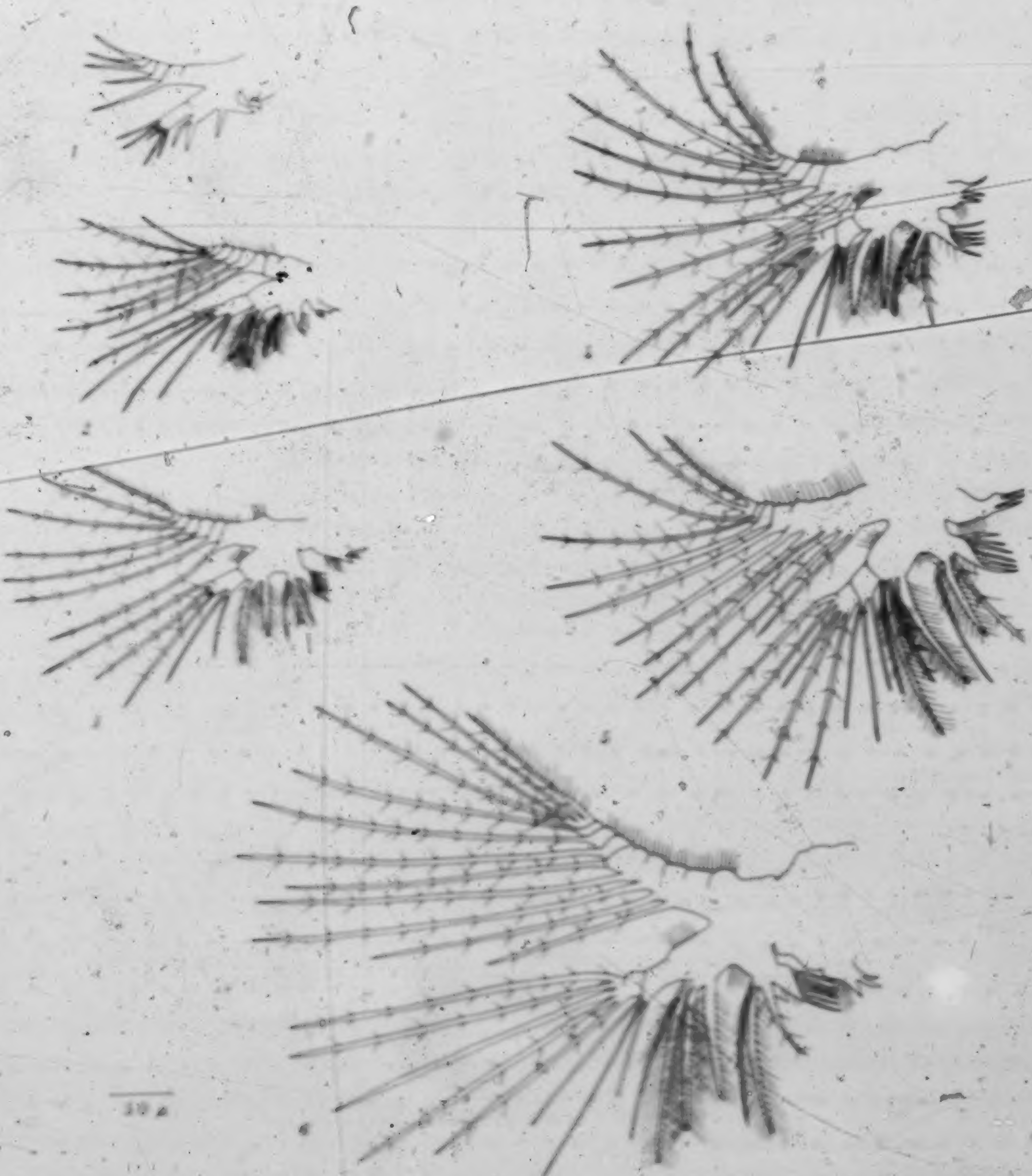


Table 20. Pollicipes polymerus. *Setation formulae of the 6 naupliar stages based on the Newman (1965) type notation. S: simple seta; S': simple seta, sometimes plumose; P: plumose; P': plumose seta, sometimes simple; S: simple seta longer than half the length of the longest seta in that appendage segment; s: simple seta shorter than half the length of the longest seta in that appendage segment;* $\bar{S}$ and $\bar{s}$: *simple setae of intermediate or variable length noting the length observed most often; F: feathery seta; H: hispid seta; G: gnathobase; (s): simple seta which may or may not be present.*

Stage	Antennule	Antenna		Mandible	
		Exopodite	Endopodite	Exopodite	Endopodite
6	$\bar{S}$:P:P:PPSS:SP:P:Ps:S	p3P:7Pp	PP$\bar{S}$PP(s):SSs(s):SFFF:SFPH:G	P:5P	4S$\bar{S}$:$\bar{S}$sPP:sPCP:PPP:G
5	s:S':P:PPSS:SP:s:P :S	2pP:7Pp	PPsPP :SSs :SFPF:SFPH:G	P:5P	SSsSs:SsPP:sPCP:PPP:G
4	S:P:PPSS:SP: :P :S	pP:6Pp	PPsPs :SSs : FsF:SFPH:G	P:4P	SSsSs:S PP:sPCP:PPP:G
3	S:P'SSS:SP: :P :S	pP:5P	PP'sP :SS : F F:SFSH:G	P:3Ps	SS Ss:S PS: PCP:PPP:G
2	S'SSS:SP: :P :S	sP:P'3PS	PS' S :S$\bar{s}$: F F:SF H:G	P:3Ps	SS S :S P : PCP:P P:G
1	sSSs:SS: :S :S	_S:4S	SS s :S$\bar{s}$: S S: S S:G	S:3S	SS s :S S : SS :S S:G

gnathobase in *P. polymerus* (Fig. 30). The same distribution of these setal types occurs in *Chthamalus aestuarii* (Sandison, 1967). The notation of variability of setal type employed by Newman (1965) is adopted here; i.e., *S'* = a simple seta sometimes plumose, *P'* = plumose sometimes simple). It has been recognized that relative setal length is difficult to assess and has sometimes been omitted from larval descriptions (Sandison, 1967; Molenock and Gomez, 1972). However, all the available data regarding the larval description are given here. *S* refers to a simple seta longer than half the length of the longest seta in that appendage segment, *s* refers to one shorter than half the longest seta in that appendage segment, and $\overline{S}$ and $\overline{s}$ to setae of intermediate or variable length, noting the length observed most frequently. Moreover, developmental intermediates where variability in the number of setae was observed have been described for some species (Norris *et al.*, 1951). In the antennary endopodites of some Stage 6 *P. polymerus* nauplii, 2 small, simple setae may or may not be present. The notation used in this case is (*s*).

Three cases exist in which setal sequence is interpreted as adjacent although the setae lie in different planes. For example, in the 2 antennary endopodite segments preceding the gnathobase of Stage 6 nauplii, the sequences are interpreted as: *SFFF* and *SFPH*. The first and third setae lay in Plane 1 and the second and fourth setae lay in Plane 2. This is also the case for the mandibular endopodite segment *sPCP*.

Cypris larva and settlement. Cypris larvae of *Pollicipes polymerus* are very similar to cyprids of other barnacles morphologically, except that the large frontal oil droplets observed in other species are not prominent in *P. polymerus* larvae (Fig. 31). Therefore, these larvae may normally feed or be extremely short-lived in the plankton.

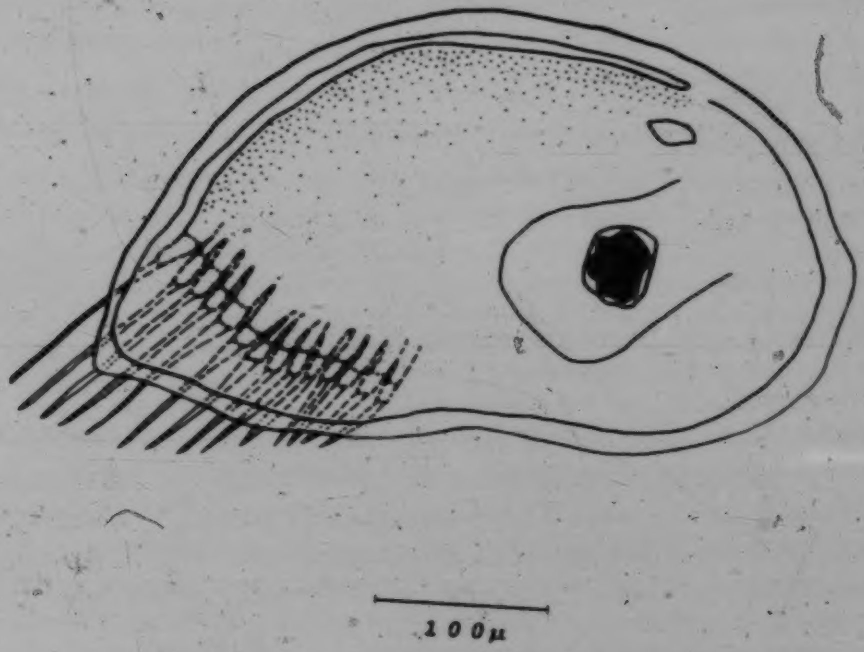
No cyprids settled on etched (n = 20) or smooth (n = 20) glass slides, whether or not bacterial films were present. When presented with only a piece of mudstone from Duxbury Reef, California, no settlement occurred (n = 20). Bubbling air into the settling beakers did not induce settling (n = 20). Two cyprids did settle on mudstone rock closely adjacent to the base of an adult *Pollicipes polymerus* and one settled on the base of the peduncle of a healthy adult (n = 10). No cyprids settled on any other substrate presented to them (peduncle epidermis and slides dipped in *P. polymerus* adult extract), nor did *P. polymerus* extract alone stimulate settlement. It is also interesting that settled juveniles were never found far from established adult clusters in the field. From 37 adult clusters, an average of 81% of the associated juveniles preferred the adult peduncle to any other available substrate.

An estimate of larval dispersion from Bodega Bay, California, was made knowing the average current speeds in that area (0.1 to 0.5 knots) and knowing that it takes an average of 42 days to reach the cypris stage from hatching at 12°C *in vitro* when fed *Prorocentrum micans/Platymonas* sp. algae (Lewis, 1975a). Theoretically, *P. polymerus* larvae could disperse from up to 116 to 580 miles.

Discussion

The 6 naupliar stages of *P. polymerus* may be distinguished from each other by the following key:

Figure 31. *Pollicipes polymerus*. Outline drawing of the cypris larva (lateral view).


100µ

1) 180 to 230 μ long, shape triangular, horns point
posteriorly .... Stage 1

2) Greater than 285 μ long, goblet-shaped, horns approximately perpendicular to body
   a) without carapace 3
   b) with carapace 4

3) a) One pre-axial antennulary seta .... Stage 3
   b) without pre-axial antennulary setae .... Stage 2

4) a) Six pairs of Series 2 spines on abdominal process;
   penultimate antennular segment swollen .... Stage 6
   b) No such spines or swelling 5

5) a) Three pre-axial antennulary setae; 1 pair
   Series 3 spines on abdominal process .... Stage 5
   b) Two pre-axial antennulary setae; no Series 3
   spines on abdominal process .... Stage 4

All balanoid nauplii appear to have trilobate labra (Molenock and Gomez, 1972), thus *P. polymerus* nauplii may be distinguished by their unilobate labra.

The setation formula of *P. polymerus* (Table 20) illustrates development of setal elaborations during naupliar development. It can be observed that a simple (*S*) seta may become plumose (*P*), feathery (*F*), combed (*C*), or hispid (*H*). In addition, a simple seta becomes plumose on the antennal endopodite of Stage 5 and, by Stage 6, it is feathery. No setae or their elaborations are lost after they develop.

A Stage 6 "developmental intermediate" (Norris *et al.*, 1951) has been described, having 2 fewer setae on the antennal endopodite than other Stage 6 larvae. Very few of the specimens examined had the 2 additional setae and no other consistent morphological differences were observed in relation to their presence.

P. polymerus naupliar setation is very similar to that of *Chthamalus aestuarii* (Sandison, 1967). The 2 newly-defined setal types found in the Chthamalidae (Bassindale, 1936; Sandison, 1954, 1967) have now been observed in the Lepadidae and cannot be considered a family character. *Mitella (= Pollicipes) mitella* also appears to have a hispid seta on the antennary endopodite, at least through Stage 4 (Yasugi, 1937) and setation is similar to that in *P. polymerus* nauplii. However, *P. spinosus* nauplii have fewer setae than *P. polymerus* at Stage 6 and no hispid or feathery setae are identifiable from the illustrations of Batham (1946). *P. polymerus* nauplii resemble *C. aestuarii* and *Balanus pallidus stutsburi* nauplii (Sandison, 1967) in that their abdominal processes grow longer than their caudal spines with succeeding larval stages.

Lepas fascicularis nauplii have ornate, spiney carapaces and longer, thinner abdominal processes in relation to their carapace length (Willemoes-Suhm, 1876) than do *P. polymerus* nauplii. *L. pectinata*, *L. anatifera* (Moyse and Knight-Jones, 1967), *L. fascicularis* (Willemoes-Suhm, 1876) and *Mitella (= Pollicipes) mitella* (Yasugi, 1937) nauplii are much larger than *P. polymerus* nauplii at any given stage, and *P. spinosus* (Batham, 1946) is larger than *P. polymerus* at Stage 6 and probably at all other stages. *M. mitella* nauplii (Yasugi, 1937) have 2 long carapace spines which are not observed in *P. polymerus* or in *P. spinosus*. (Batham, 1946).

Cypris larva settlement was stimulated only when healthy adult peduncles were available. Pomerat and Weiss (1946) showed that surface porosity and fibrous nature of the surface influenced some barnacle settlement. Crisp and Barnes (1954) and Barnes (1955a, 1956) found cyprids very sensitive to surface contours, while Knight-Jones (1953) and Crisp and Meadows (1962, 1963) felt that cyprids responded to contact with some cuticular substance in conjunction with a chemical settling factor ("contact chemical sense"). Sensory structures on the cypris antennule of *Balanus balanoides* have been described (Nott, 1969; Nott and Foster, 1969; Gibson and Nott, 1971). Barnes and Reese (1960) suspected that *P. polymerus* cyprids react to a specific property of the adult peduncle. From the present study it appears that both chemosensory and tactile responses are necessary for the specific settling behavior of *P. polymerus* cyprids.

PERISTALTIC CONSTRICTIONS IN THE FERTILIZED EGG[7]

Introduction

This chapter deals with aspects of peristaltic constriction as a special developmental phenomenon.

During the maturation divisions of the fertilized barnacle egg, an unusual event occurs: a series of constriction rings move slowly from the animal to the vegetal pole. This process, here called peristaltic constriction, was noted at the turn of the last century (Nussbaum, 1890; Groom, 1894; Bigelow, 1902) but has been overlooked by a number of contemporary barnacle embryologists (Batham, 1946; Anderson, 1969; Walley *et al.*, 1971). As far as is known, no comparable process has been reported in other animals except perhaps in the eggs of a salamander (Hara, 1971), an insect (Vollmar, 1972), and a shrimp (Rappaport, 1960).

Microfilaments (40 to 70 Å diameter) have been called the "agents of cellular contraction" (Schroeder, 1971). Changes in the organization of microfilaments or the appearance of arrays of microfilaments have been correlated with specific changes in cellular shape during ascidian tail resorption (Cloney, 1966, 1969), neurulation in amphibians (Baker, 1965; Baker and Schroeder, 1967; Schroeder, 1970a; Karfunkel, 1971; Burnside, 1971, 1973), and the development of various organs (see Wessells *et al.*, 1971, for a review). Microfilaments form a contractile ring associated with the cleavage furrow in dividing HeLa cells (Schroeder, 1970b), marine invertebrate eggs (Arnold, 1968, 1969; Goodenough *et al.*, 1968; Schroeder, 1968, 1969, 1972; Szollosi, 1968, 1970; Tilney and

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[7] Portions of this chapter are extracted from a paper in print: LEWIS *et al.*, 1973.

Marsland, 1969) and vertebrate eggs (Bluemink, 1970, 1971; Selman and Perry, 1970; Kalt, 1971; Gulyas, 1973). Also, microfilaments have been implicated in particle movement in various systems (Nagai and Rebhun, 1966; Buckley and Porter, 1967; Pickett-Heaps, 1967; Rebhun, 1967; McGuire and Moellmann, 1972). One report has implicated microfilaments in the formation of repetitious peristaltic contractions in adult ascidian ampullae (De Santo and Dudley, 1969). As far as is known there is only 1 report on the fine structure of barnacle eggs (Woods, 1969).

Peristaltic constriction ring movement in barnacle eggs is stopped and the amplitude of constriction is reduced by treatment with cytochalasin B (CCB), but occurs in the presence of colchicine (Lewis *et al.*, 1973), which disrupts microtubules but not microfilaments (Malawista *et al.*, 1968; Weisenberg *et al.*, 1968). It was, therefore, tentatively proposed that peristaltic constriction depends upon microfilament function (Lewis *et al.*, 1973). Microfilaments (40 to 70 Å) have been shown to be sensitive to CCB treatment in most cases. Although the mechanism of action of CCB is controversial (e.g., Kletzien *et al.*, 1972; Plagemann and Estensen, 1972; Zigmond and Hirsch, 1972a,b), it may block cell movements by disorganizing thin microfilament arrays in many contractile systems (Schroeder, 1970; Wessells *et al.*, 1971), but it does not affect microtubules.

The purposes of this chapter are: 1) to measure some dynamic parameters of peristaltic constriction by using time-lapse cinematography, 2) to obtain some evidence of its causal mechanism by using different chemical inhibitors, 3) to document the presence and orientation of organelles, especially microfilaments (45 to 60 Å diameter) associated with constriction rings, and 4) to suggest a model to explain this morphogenetic movement.

Materials and Methods

Adult *Pollicipes polymerus* were collected from rocky shores on San Juan Island, Washington, at low tide. Freshly fertilized eggs were dissected out from the mantle cavity of adults and were pipetted into filtered and UV-light treated sea water in watch glasses at the ambient sea water temperature (13° to 15°C) and fixed when the desired post-fertilization stage (first polar body through cleavage) was observed.

Time-lapse films were made with a Bolex H16M camera and cinephotomicrographic apparatus (Sage Instruments) coupled to a Zeiss Universal microscope with Nomarski differential interference contrast optics. Ambient sea water temperature was maintained with a thermoelectric cooling stage (Cloney *et al.*, 1970). An L and W photo-optical data analyzer was used for film analysis.

Batches of eggs were treated with: 0.1 to 20 $\mu g/cm^3$ CCB (dissolved in dimethylsulfoxide) for up to 140 min and 0.4 to 400 $\mu g/cm^3$ colchicine, 10 to 20 $\mu g/cm^3$ cycloheximide, 5 to 10 $\mu g/cm^3$ actinomycin D, or 550 $\mu g/cm^3$ antimycin A (dissolved in 0.95% ethanol) for up to 90 min. Appropriate controls were carried out by using sea water, 0.2% v/v dimethylsulfoxide and 0.95% ethanol.

Eggs were fixed for transmission electron microscopy 30 min each with Millonig's (1961) phosphate-buffered 2.5% glutaraldehyde, pH 6.6 (Dunlap, 1966; Cloney and Florey, 1968), followed by phosphate-buffered 1% OsO_4; or cacodylate-buffered 2% glutaraldehyde with ruthenium red (Luft, 1971; modified by M. Cavey, Personal communication) followed by phosphate-buffered 1% OsO_4, all at room temperature. The ruthenium red fixation was found to heighten background electron density such that it eliminated much of the contrast necessary to demonstrate microfilaments and microtubules. On the other hand, phosphate-buffered glutaraldehyde fixatives revealed these structures better. 2.67% OsO_4 buffered with s-collidine (Bennett and Luft, 1959); s-collidine-buffered 2.5% glutaraldehyde, pH 6.7, followed by phosphate-buffered 1% OsO_4; veronal acetate-buffered 1% OsO_4 (Palade, 1952); and 2.5% glutaraldehyde/1% OsO_4 cocktail, pH 6.6, gave unsatisfactory results. All micrographs in this paper are of eggs fixed with phosphate-buffered glutaraldehyde/OsO_4.

Following ethanol dehydration, specimens were transferred through 2 to 3 changes of propylene oxide over a total period of 15 min. Eggs were next infiltrated in 1 part propylene oxide to 1 part Epon 812 (Luft, 1961) for 24 h, then transferred to the embedding medium and polymerized in a 60°C oven for 1 to 2 days or at 80°C for 3 h.

Sections for light microscopy were cut at thicknesses of 0.5 to 1.0 µ and stained with methylene blue and azure II (Richardson *et al.*, 1960). Silver to gray (600 to 900 Å) sections for electron microscopy were cut with glass knives (LKB knifemaker) or a Dupont diamond knife on a Sorvall Porter-Blum MT-2 ultramicrotome and collected on acetone-cleaned 200 mesh grids coated with Formvar film. Sections were doubly stained from 30 min to 2 h with 2% (saturated) aqueous uranyl acetate at room temperature or for 10 min with 1% aqueous uranyl acetate at 55° to 60°C (Chang, Personal communication), followed by 4 to 10 min with lead citrate (Reynolds, 1963). Observation and electron micrography was with an RCA EMU-3F electron microscope operated at 50 KV or a Phillips 200 electron microscope operated at 60 or 80 KV.

For scanning electron microscopy (SEM), eggs were teased from the ovigerous lamellae as the fertilization membrane was raising and were placed in a clean watch glass. The fertilization membranes were removed by allowing them to stick to the watch glass and then gently agitating the eggs so that the fertilization membranes were left behind. The tissue was fixed when peristaltic constriction rings were seen, but before the egg membrane fully raised. The egg membranes were not removed. Eggs were fixed at room temperature in Millonig's phosphate-buffered 2% glutaraldehyde for 1 h, rinsed and post-fixed in phosphate-buffered 1% OsO_4 for 30 min, dehydrated and critical point dried. They were carbon and gold coated (75 to 100 Å, each) in an Edward's vacuum evaporator and viewed with a Cambridge S-4 stereoscan scanning electron microscope.

Various organelles were observed and counted in eggs sectioned longitudinally from photomicrographs at a magnification of X37,500. Constriction ring and adjacent non-constriction ring areas in Stage III eggs (Lewis *et al.*, 1973) were contrasted by comparing average numbers of organelles using the *t*-test (Sokal and Rohlf, 1969). It should be noted that because of the small sample size and large variance of the samples, the *t*-test as used here demonstrates significance of only large differences between means.

Observations and Results

Stages of Constriction

Although variability in the rate of embryonic development in different eggs is considerable, normal development from the beginning to the completion of peristaltic constriction is divided here into 5 stages. Stage I: 1.5 h after insemination; egg spherical; 1, then 2 constriction rings; ooplasm visibly homogeneous. Stage II: 2 h after insemination; egg still spherical or beginning to elongate; 2 constriction rings; ooplasm visibly homogeneous; first polar body forms. Stage III (Fig. 32): 2.2 h after insemination; elongation of the egg into an ovoid shape; 3 to 5 constriction rings; beginning of ooplasmic segregation (shifting of large yolk platelets to the vegetal half); initial elevation of egg membrane from the egg surface. Stage IV: 4 to 6 h after insemination; further elongation of the egg; 1 to 2 constriction rings; further segregation of ooplasm; further lifting of the egg membrane. Stage V: 4 to 6 h after insemination; further elongation of the egg; peristaltic constriction stops; ooplasmic segregation nearly complete; egg membrane elevated; second polar body forms.

Cine Film Analysis[8]

The results of the microcinematographic film analysis, as shown in Figures 33 and 34, illustrate a number of points of peristaltic constriction. One sees in Figure 33 that it takes about 8.5 min for a constriction ring to travel about 85% of the egg's length (total

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[8] Time lapse film is deposited in the Extension Department, University of Alberta, Edmonton, Alberta, Canada.

Figure 32. *Pollicipes polymerus*. Stage III egg showing 3 constriction rings moving from the animal to the vegetal pole in a 120 sec sequence (A to D). a, animal pole; v, vegetal pole; fm, fertilization membrane; em, egg membrane.

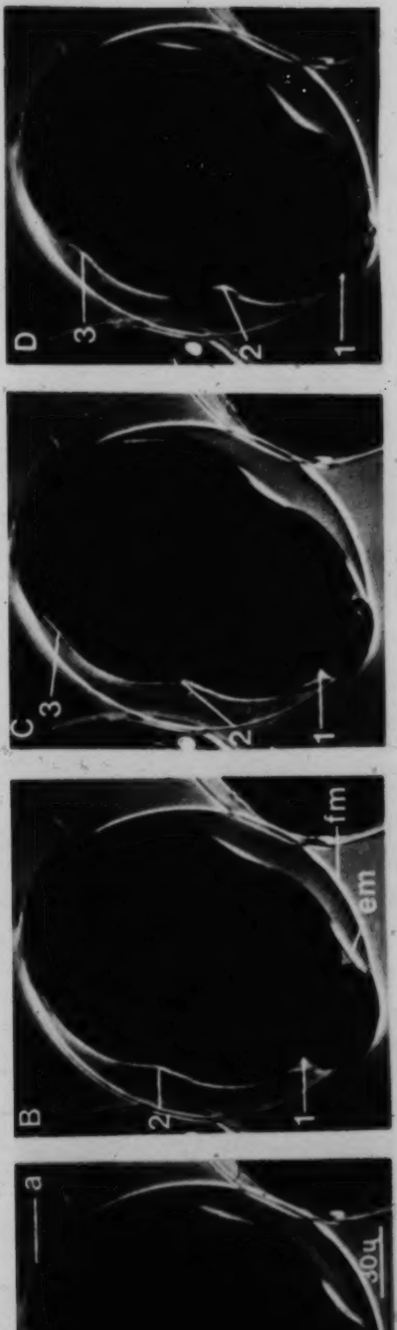

A
2
1
v
a
30μ
B
2
1
em
fm
C
3
2
1
D
3
2
1

Figure 33. *Pollicipes polymerus*. Peristaltic movement of constriction rings measured in different developmental stages (I to V) as a function of time. The number in parentheses following the developmental stage refers to the number of constriction rings measured for that curve. The time the constriction ring ends vegetally was chosen as the reference time.

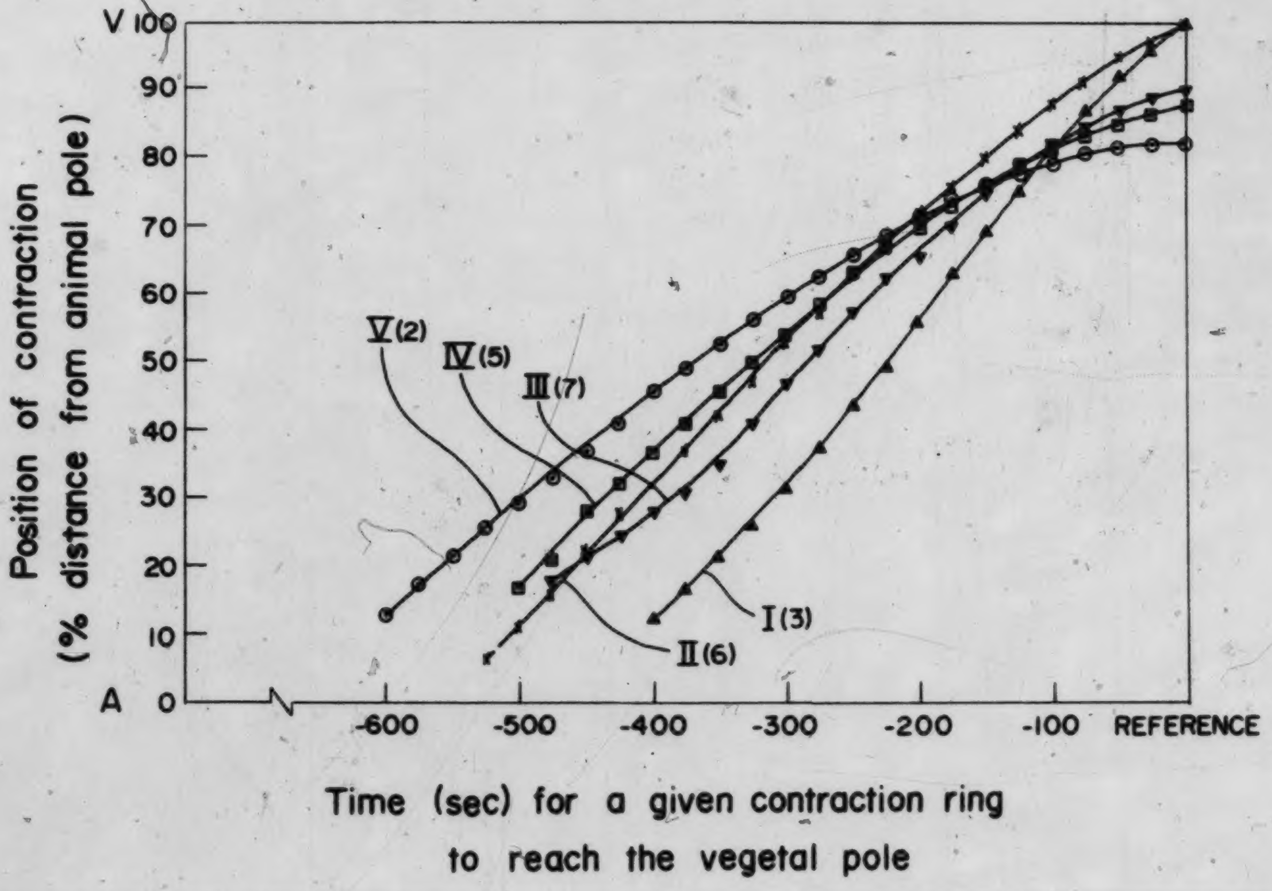
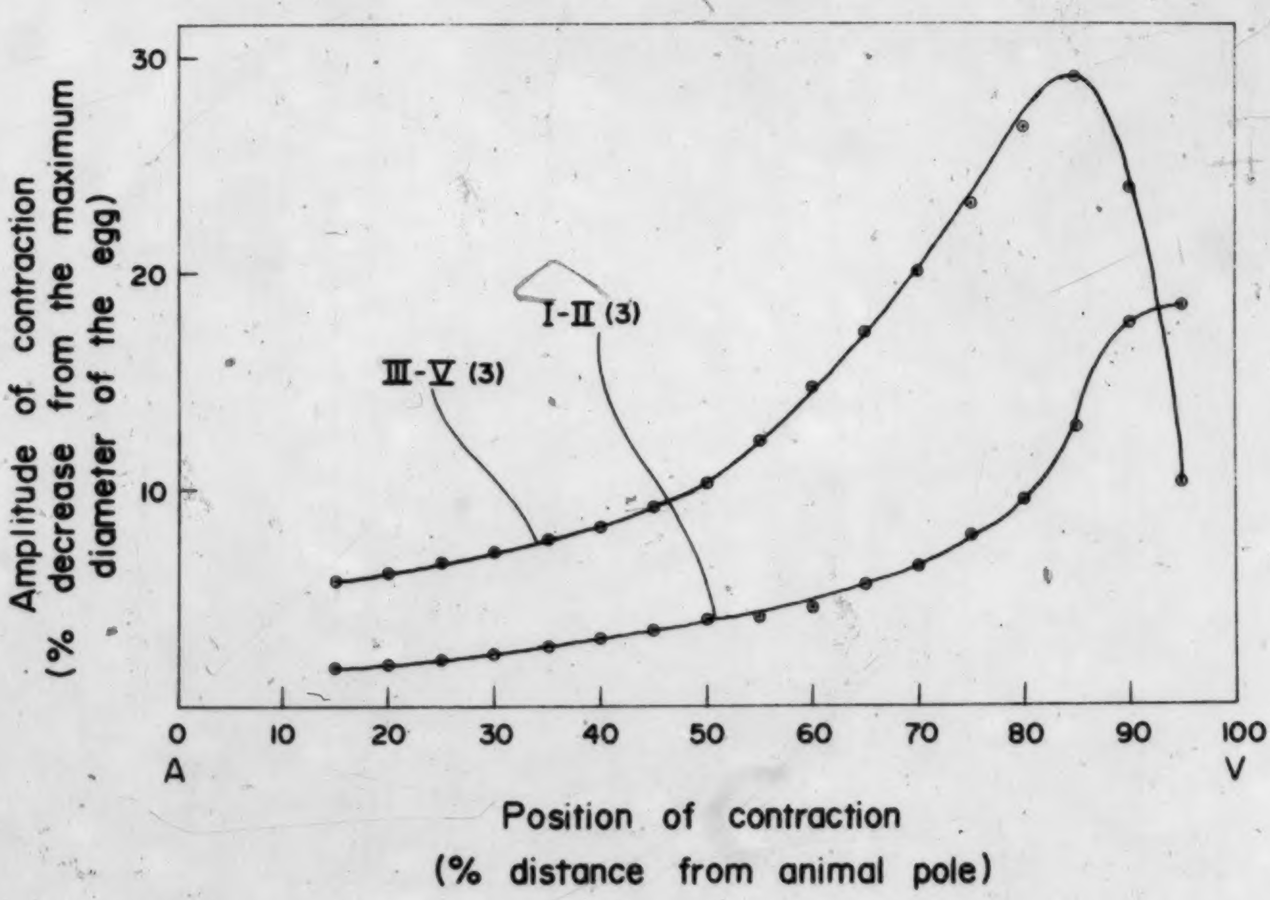

V 100
90
80
70
60
50
40
30
20
10
A 0
Position of contraction
(% distance from animal pole)
-600
-500
-400
-300
-200
-100
REFERENCE
Time (sec) for a given contraction ring
to reach the vegetal pole
V (2)
IV (5)
III (7)
II (6)
I (3)

Figure 34. *Pollicipes polymerus*. Amplitude (average) of constriction in eggs of different developmental stages (I to V) as a function of the constriction position on the animal-vegetal axis. The number in parentheses following the developmental stage refers to the number of eggs measured for that curve.


30
20
10
Amplitude of contraction
(% decrease from the maximum
diameter of the egg)
III-V (3)
I-II (3)
0
10
20
30
40
50
60
70
80
90
100
A
V
Position of contraction
(% distance from animal pole)

length is 140 μ). Figure 33 shows also that the constriction rings travel faster at the animal half than at the vegetal half and the velocity of the peristaltic motion decreases with embryo age; constriction rings at Stages I and II travel right to the vegetal tip, whereas rings at Stages III to V stop at increasing distances from the vegetal tip.

The amplitude of the constriction changes in relation to developmental stage as well as the position of the constriction in the egg (Fig. 34). This figure shows that the amplitudes of the constriction are greater in older embryos (III to V) than that of younger ones (I and II). Moreover, the amplitude of constriction of all stages increases generally from animal to vegetal pole but drops sharply at the vegetal pole in older stages (III to V).

The rates of peristaltic motion in different embryos seem to be independent of the number of rings present. For example, the interval between constriction rings reaching the vegetal pole is 3 min in an egg with 3 rings, and the same amount of time is required in an egg with 4 rings.

Effect of Some Chemical Inhibitors

Chemical inhibitors used in this study which may affect the peristaltic constrictions include: CCB (in dimethylsulfoxide), colchicine, actinomycin D, cycloheximide and antimycin A. CCB is known to inhibit some cellular contraction (Carter, 1967; Schroeder, 1969, 1970b; Bluemink, 1971a) although the mechanism of its action is controversial. Some experiments indicate CCB disrupts contractile microfilaments (Schroeder, 1968; Lash *et al.*, 1970; Wessells *et al.*, 1971; Schroeder, 1972), whereas others show it appears to affect membrane phenomena (Bluemink, 1971a,b; Krishan, 1971). In this study it was found that 0.25 to 20 μg/cm^3 CCB in 0.025 to 0.2% dimethylsulfoxide effectively stopped and dampened all peristaltic constrictions within 2 to 30 min. Colchicine, a known microtubule inhibitor (Eigsti, 1947), produced no effect on peristaltic constriction at concentrations up to 400 μg/cm^3 for 80 min but both ooplasmic segregation and meiotic division were inhibited; 4 μg/cm^3 did not affect ooplasmic segregation, but apparently arrested mitosis, for cleavage failed to occur. Actinomycin D, an inhibitor known to block amino acid transport (Yamada *et al.*, 1967), stimulate mRNA degradation (Kennell, 1964), and inhibit protein (Honig and Rabinovitz, 1965) and phospholipid synthesis (Pastan and Friedman, 1968), slowed the rate of peristaltic motion slightly at concentrations of 5 to 20 μg/cm^3, but did not stop it. Ooplasmic segregation was unaffected, but development ceased at the 2 to 4 cell stage. Cycloheximide, an inhibitor of protein synthesis (Pestka, 1971), slowed the peristaltic motion slightly at concentrations of 10 to 20 μg/cm^3 but did not affect ooplasmic segregation. Antimycin A, an inhibitor of the cytochrome oxidase cycle in mitochondria, and therefore of energy release (Roodyn, 1967; Green and Baum, 1970), did not inhibit the constrictions at 550 μg/cm^3 but arrested ooplasmic segregation.

Peristaltic Constriction Rings in Temporal Perspective

Pollicipes polymerus eggs are oviposited into a sac produced by the oviducal gland which holds the egg mass together in the mantle cavity throughout the brooding period (Walley *et al.*, 1971). The eggs are fertilized here. Soon after sperm entry, the fertilization membrane elevates quite far from the zygote (Fig. 35A). Eggs are crowded in the lamella sac, so that fertilization membranes of adjacent eggs adhere to each other (Fig. 35B). Within a few minutes after the fertilization membrane is raised, the first polar body forms at the animal pole (Figs. 35A and C) (see also Lewis, 1975b, for details in development). The fertilization membrane consists of a loose fibrogranular matrix, similar to the vitelline membrane in *Lymnaea* (Morrill and Perkins, 1973), and appears relatively uniform in thickness (mean diameter is 0.15 μ). Extracellular to both zygote and polar body are dense plaques (Figs. 35C, 36C and D), such as those which Anderson *et al.* (1970) found during the first appearance of the tertiary envelope of fertilized *Artemia salina* eggs. Microvilli appear at the animal pole when the first polar body lifts (Fig. 35C), but nowhere else on the egg's surface, except at constriction rings. Dense bodies (65 to 100 mμ) which appear to be derived from nearby Golgi complexes (Fig. 36D) are localized subjacent to the cleavage furrow and remain in the cortex of both polar body and zygote (Figs. 36B and C). These membrane-bound dense bodies have a substructure of circular or uneven material (Fig. 36B). In Figure 36A, just below the microvilli and remnants of an intercellular bridge, a 45 Å microfilament is observed lying in the former cleavage plane. Moreover, to either side of the polar body, an area of microfilamentous meshwork is observed, with filaments running into the microvilli (Fig. 36C).

Vesicles are observed around the chromatin masses in both zygote and first polar body (see also, Babbage and King, 1970), but it is not clear whether the vesicles completely fuse in either cells of *P. polymerus*. Besides the aforementioned organelles, only a few, small, scattered

Figure 35. *Pollicipes polymerus.*

A. Longitudinal section of a fertilized egg with the first polar body (PB) during cleavage. The fertilization membrane (FM) is raising and a single peristaltic constriction ring (indentation) is visible. X 1,780.

B. Longitudinal section of a fertilized egg with the first polar body (PB) attached. Several eggs are viewed, as this section was cut from an egg mass. The fertilization membranes adhere to one another (double arrow) and a lightly-stained material is observed between the oolemma and the fertilization membrane. Egg contours are molded by adjacent eggs. X 2,080.

C. Longitudinal section of a first polar body. Numerous dense bodies (DB) occur along the cleavage furrow in both egg and polar body. Microvilli (V), dense extracellular plaques (P), many microtubules and the fertilization membrane (FM) are visible. X 19,400.

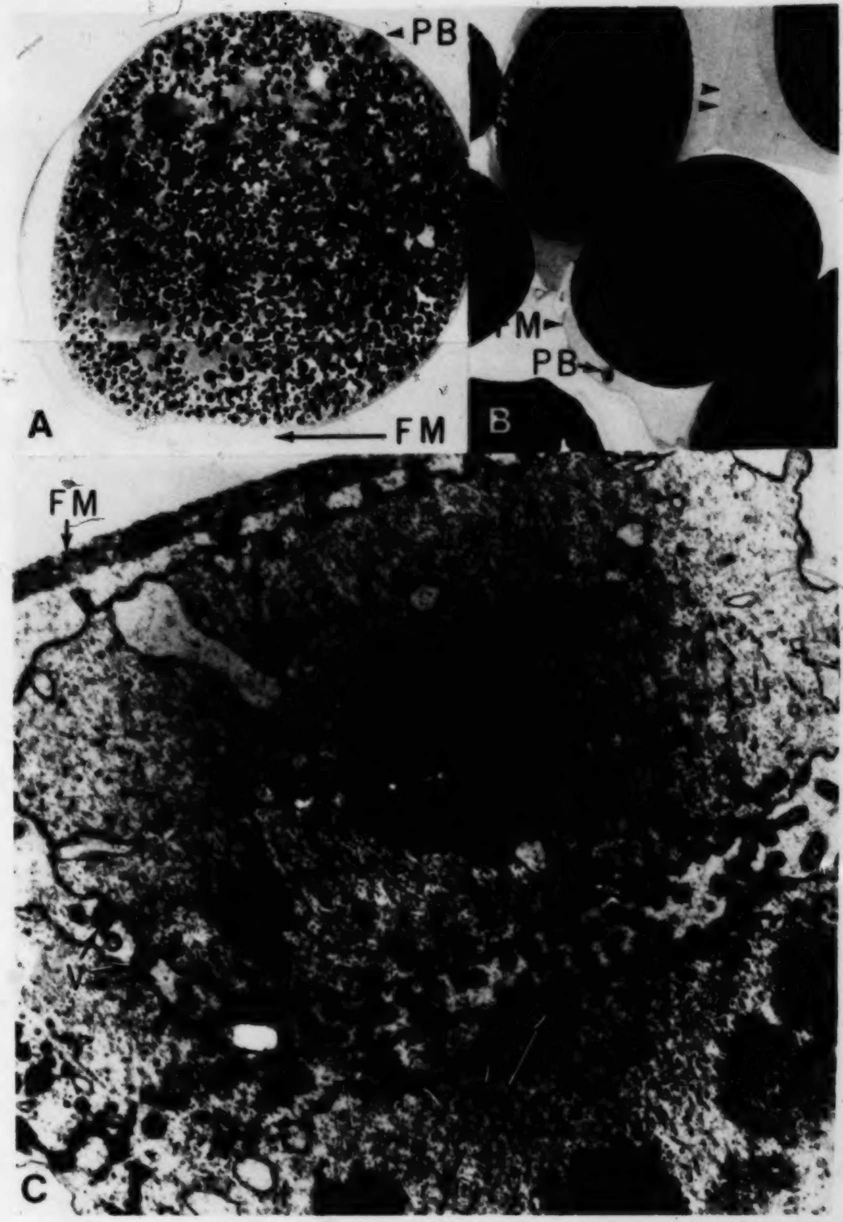

PB
A
FM
FM
PB
B
FM
V
C

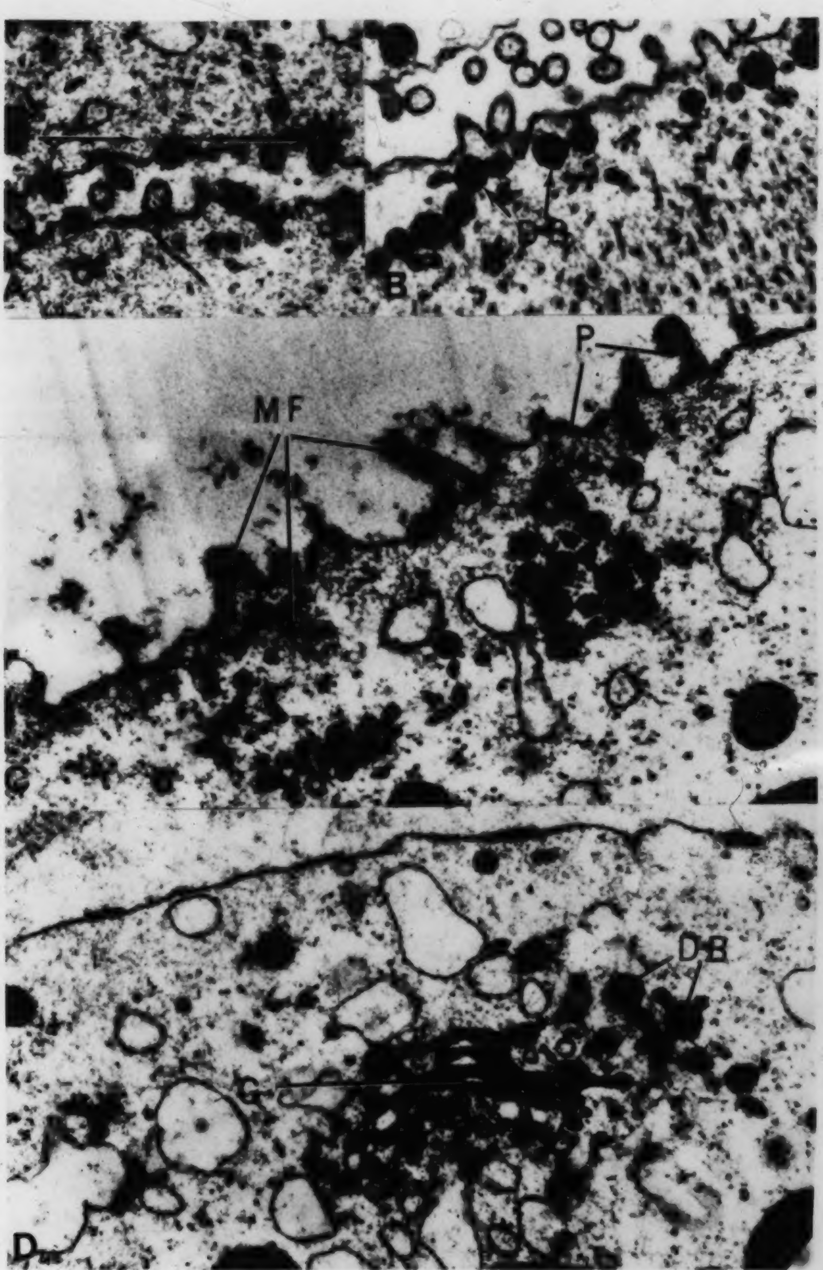
Figure 36. *Pollicipes polymerus.*

A. Longitudinal section of the animal pole where the first polar body has just cleaved. A 45 Å diameter microfilament (arrows) is oriented in the plane of cleavage, possibly remnant from a contractile ring. X 28,480.

B. A higher magnification view of dense bodies (DB) in the animal pole along the cleavage furrow, showing their substructure of alternating light and dark material surrounded by a membrane. Microtubules are also apparent. X 33,370.

C. Longitudinal section of the animal pole adjacent to the first polar body. Microvilli contain a network of thin microfilaments (MF). Dense bodies (DB) and extracellular plaques (P) are also apparent. X 26,320.

D. Longitudinal section of the animal pole to 1 side of where the first polar body lifted. A well-developed Golgi complex (G) is associated with the membrane-bound dense bodies (DB) in the cortex. X 26,790.


A
B
DB
P
MF
C
DB
G
D

vesicles and many spindle microtubules are observed in the first polar body.

During this period, corresponding to the time when the eggs become sticky, a material of intermediate electron density appears on the outer surface of the fertilization membrane (Fig. 38E). In *Carcinus* a fluid of similar density exuded from the egg at spawning plays an important part as a "glue" in the perivitelline space which subsequently promotes adhesion of eggs to the female's abdomen (Cheung, 1966).

When the egg membrane was inadvertently torn during removal of the fertilization membrane for scanning electron microscopy, the remaining egg membrane collapsed and this obscured the constriction ring (Fig. 37A, egg 1). When this happened the vegetal oolemma of the egg burst, the cytoplasmic contents were extruded, the rings deepened, and the egg became more elongate (compare eggs 1 and 2, Fig. 37A). This phenomenon may be due to an increase in extracellular pressure when the fertilization membrane is removed and/or a concomitant increase in force of the constriction rings. Thus, circumferential lines of stress associated with constriction rings are most clearly seen in SEM views of the vegetal pole when the egg is ruptured (Fig. 37B). The egg membrane (mean diameter is 0.06 μ) is normally lifted above the peristaltic constriction rather than following its contours (Figs. 37C and D). A thick cortex is also observed at the constricted area (Fig. 37C). During this period, there is a segregation and subsequent concentration of large lipid yolk droplets at the vegetal pole (Fig. 37C) from a previously homogeneous egg (Fig. 35A).

By the time the second polar body forms (Figs. 38A and B), the constriction rings are no longer observed. An electron dense layer

Figure 37. *Pollicipes polymerus.*

A. Scanning electron micrograph of eggs with egg membranes still adhering. The egg membrane of egg 1 is perforated and the egg is longer with deeper constriction rings than that of egg 2. X 285.

B. Scanning electron micrograph of a vegetal constriction ring in an egg with the perforated egg membrane adhering. Circumferential lines of stress are visible (arrows). X 1,100.

C. Median longitudinal section of an egg with 2 constriction rings. X 2,300.

D. Longitudinal section grazing (G) a vegetal constriction ring. Egg membrane raised at the constriction ring areas (arrows). X 3,180.

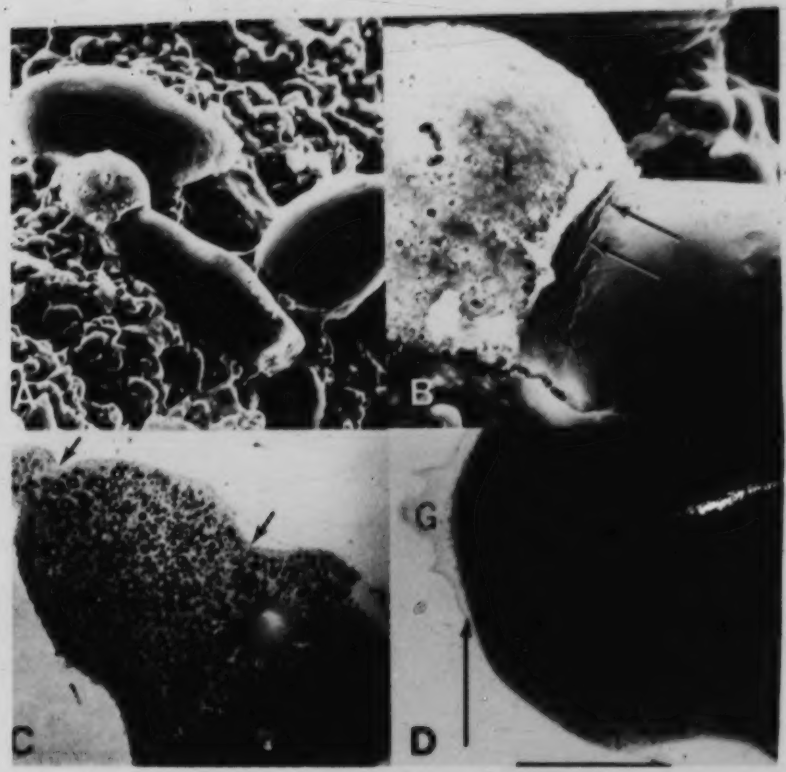

A
B
C
G
D

Figure 38. *Pollicipes polymerus.*

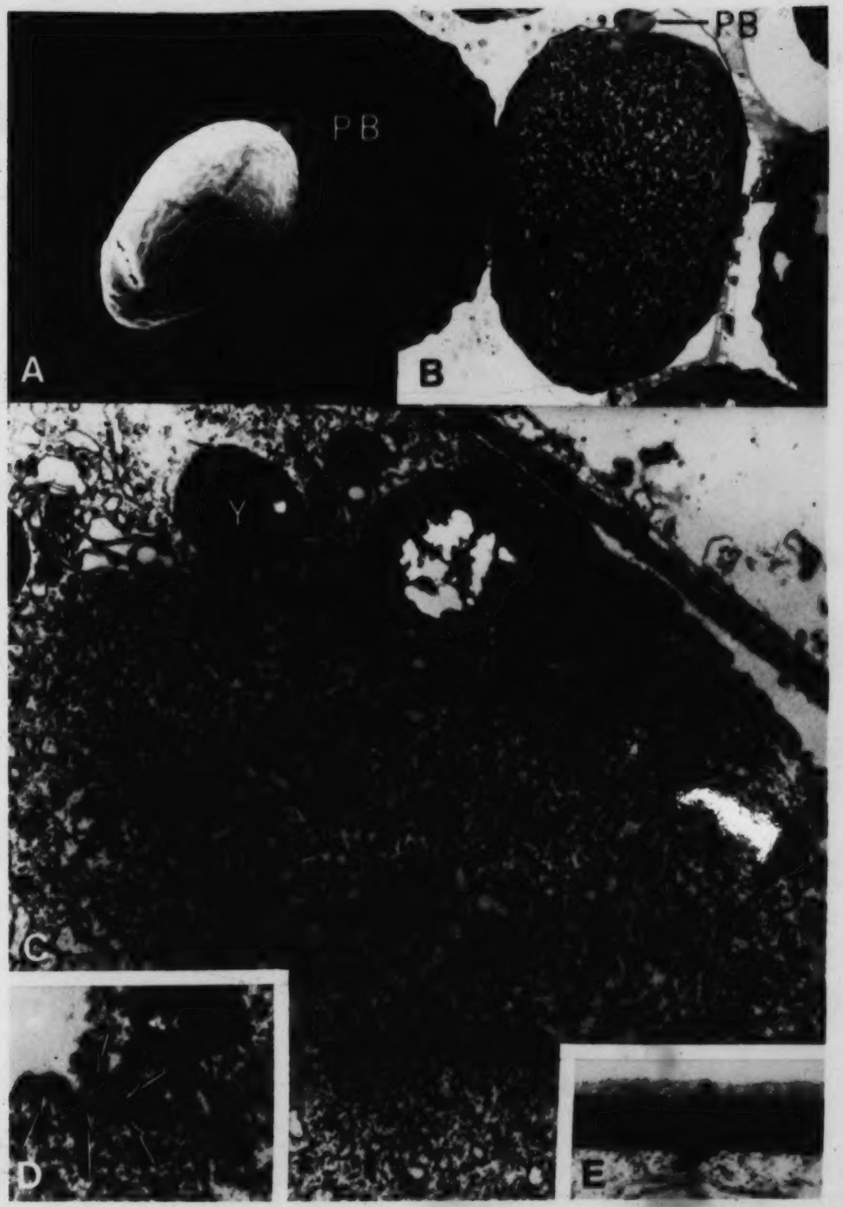
A. Scanning electron micrograph of an elongate egg with a second polar body (PB). The fertilization membrane has been removed and constriction rings are no longer visible. X 375.

B. Second polar body (PB) stage with the egg membrane lifted, egg elongated, and constriction rings stopped. X 1,960.

C. Longitudinal section of the second polar body. Some PC yolk (Y) is visible. X 20,140.

D. Second polar body cleavage furrow with dense cortical material (arrows). X 42,960.

E. Flocculent material on the outside of the fertilization membrane (asterisk). X 35,250.


PB
PB
A
B
Y
C
D
E

appears at the leading edge of the cleavage furrow during second polar body formation (Fig. 38D). The second polar body is larger (15 by 20 μ) and it contains more organelles than the first polar body (7 by 7 μ): mitochondria, yolk, vesicular endoplasmic reticulum (ER), dense 300 Å bodies identified as β glycogen granules (see Fawcett, 1966), remnant spindle microtubules and small masses of chromatin (Fig. 38C). No microvilli are seen as yet at the cytoplasmic bridge, although they will appear later. The zygote nucleus condenses and aggregates into chromatin-containing vesicles (Fig. 39A). These vesicles and dilated cisternae finally fus[illegible] to form an ovoid nucleus whose membrane has numerous pores (Fig. 39B) as in other crustacean eggs (Beams and Kessel, 1963; Hinsch and Cone, 1969).

Fine Structure of Constriction Ring Stage: General Features

Schroeder's (1970b) method and terminology of egg orientation was adopted. The various planes of section used in this study are indicated (Figs. 37D, 40 and 41A to C).

Between the oolemma and the lifting egg membrane several features appear with or without ruthenium red treatment: an extracellular layer of spheres with indistinct borders (mean diameter is 0.03 μ), some dense granular and occasionally filamentous (50 Å) material (Fig. 42A), reminiscent of the extracellular actin-like filaments described by Perdue (1973) and Szollosi (1970), and either glycogen granules or additional extracellular spheres which appear "suspended" in this space (Fig. 42B). The egg membrane is composed of a fibrogranular material possibly formed from coalescence of the plaques previously observed at the first polar body stage. The extracellular filaments are possibly material also to be included in the egg membrane.

Figure 39. *Pollicipes polymerus*.

A. Female pronucleus immediately after the second polar body formation; condensation of the nuclear material into vesicles is noted. X 6,750.

B. Nucleus after condensation of vesicles, showing nuclear pores (P). X 21,810.

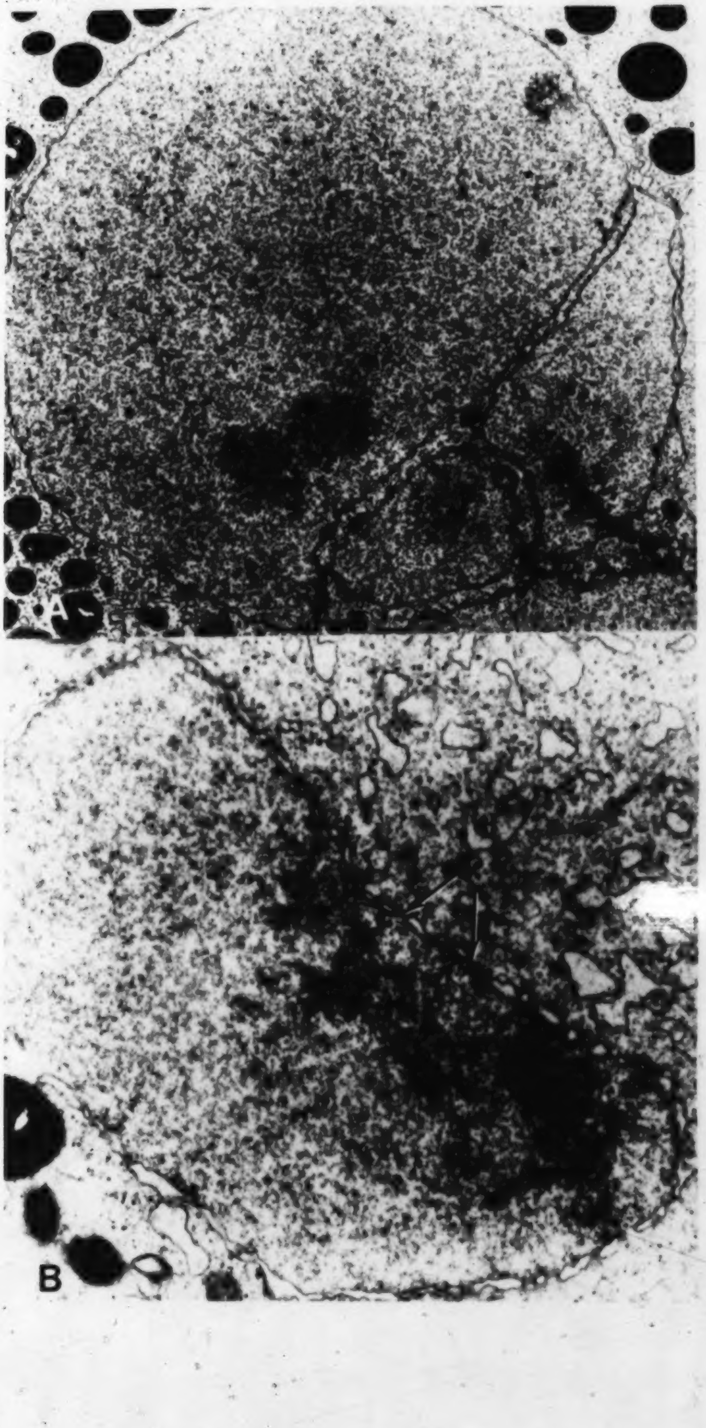

A
B

Figure 40. *Pollicipes polymerus*. Diagram illustrating the 3 planes of sectioning through an egg with peristaltic constriction rings used in this study.

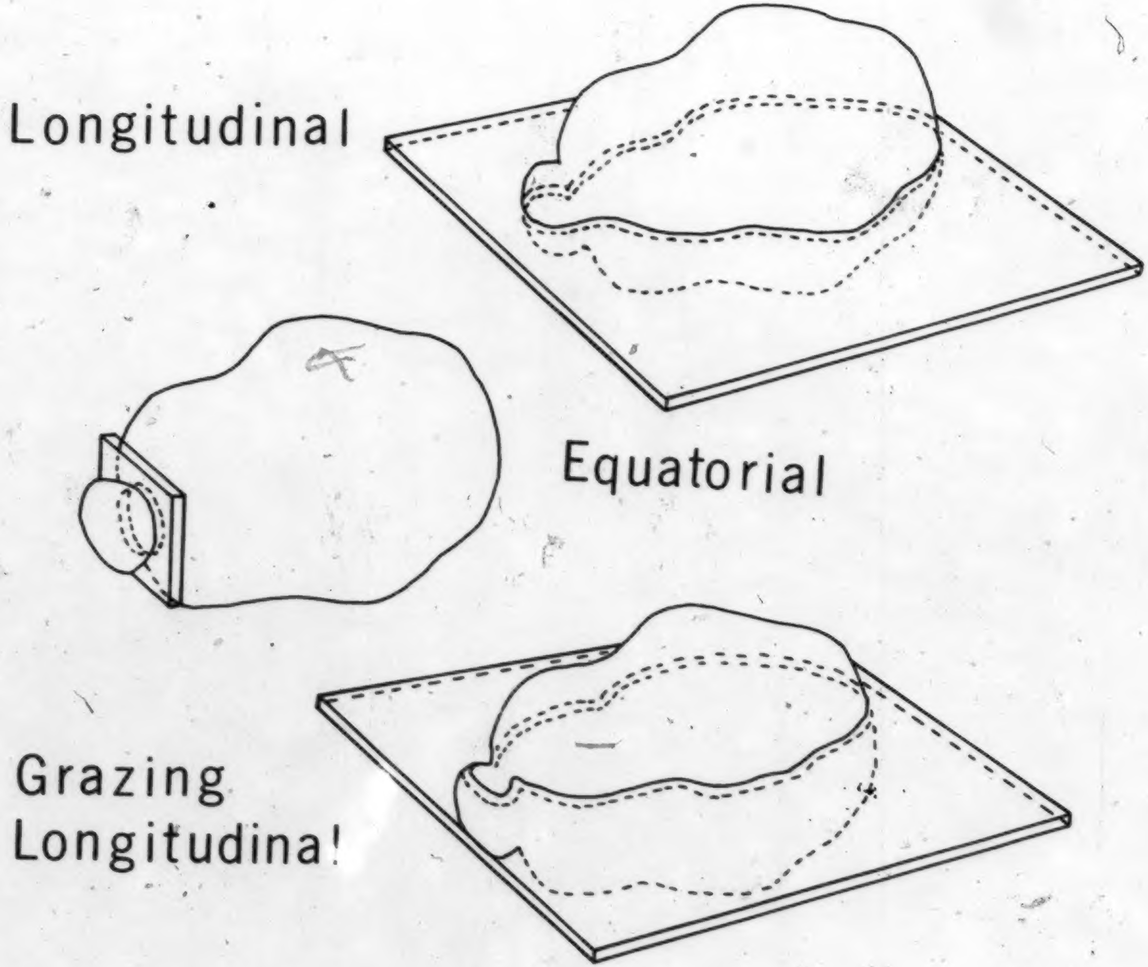

Longitudinal
Equatorial
Grazing Longitudinal

Figure 41. *Pollicipes polymerus*.

A. Equatorial view of a vegetal constriction ring. The egg membrane (EM) has elevated from the oolemma and the cortex (C) is thick. The section is slightly oblique such that about 270° is in the constriction ring. X 3,360.

B. Longitudinal section of the vegetal half of an egg with 2 constriction rings. A few large lipid yolk droplets (Y) are observed during their vegetal migration. X 2,020.

C. Longitudinal section grazing the vegetal polar region (egg membrane is raised at the constriction ring). X 2,750.

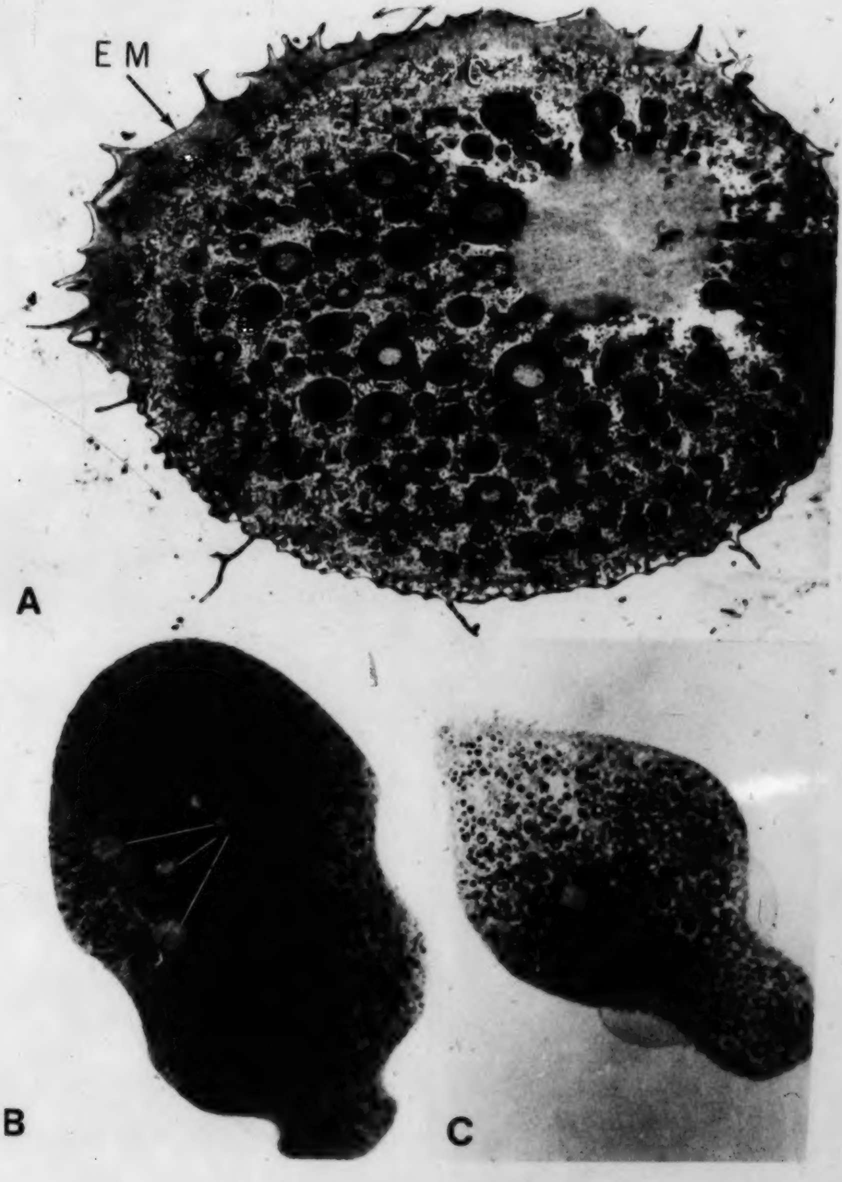
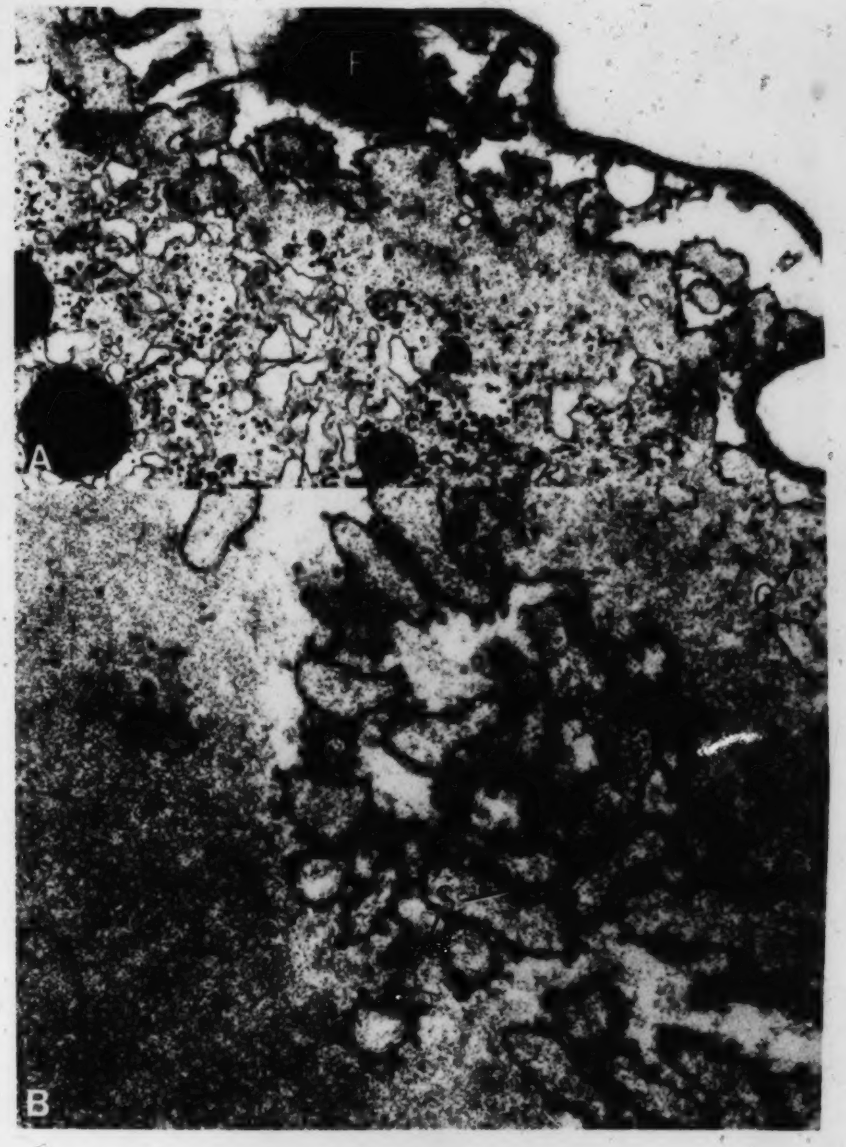

EM
A
B
C

Figure 42. *Pollicipes polymerus*.

A. Grazing longitudinal section close to a constriction ring, showing extracellular filaments (F). X 29,140.

B. Equatorial section through a constriction ring area, showing glycogen granules (G), outer border spheres (S), and extracellular flocculent material (FL). X 51,410.


F
A
B

Large granules of membrane-bound protein-carbohydrate (PC) yolk (as determined histochemically by Woods, 1969) and droplets of lipid yolk are dispersed throughout the cytoplasm. The PC yolk appears variously as granular or with central crystalloid inclusions (Fig. 43A). Membrane bound multivesiculate bodies (MVB) are also observed in several forms (Figs. 44A to F). Transparent, as well as dark and intermediate density vesicles, are enclosed within the round MVBs. The MVBs could harbor vesicles enclosing particulate yolk undergoing degradation, as evidenced by the occasional PC yolk with an indistinct outline and smooth endoplasmic vesicles enveloping it (Figs. 43A, 45A and B), instead of the single, normally well-defined limiting membrane (Fig. 45B). Several intermediate stages between dense and almost empty MVBs are also observed (Figs. 44C to E). Scattered membrane-bound, dense osmiophilic bodies (100 to 130 mμ diameter) are possibly products of the MVBs, since no Golgi complexes are observed during this stage of development (Figs. 43B, 44A and 45B) (see also Robbins and Gonatas, 1964a,b).

Smooth endoplasmic reticulum and vesicles, aggregations of deeply stained α and β glycogen granules (Perry, 1967), some ribosomes, simple mitochondria, and small dense bodies are scattered throughout the egg. Small membranous coils, sometimes referred to as myelin bodies, appear to be associated with or formed from mitochondria (Figs. 43B and C).

Although occasional scattered dense masses were seen at the animal pole, no intact nuclei were ever observed in eggs with peristaltic constriction rings.

Fine Structure of Constriction Rings

Differences in the egg contour are observed in the longitudinal sections of the animal pole, middle-position constriction ring and

Figure 43. *Pollicipes polymerus.*

A. Longitudinal section of the animal pole, showing mitochondria (M), vesicular endoplasmic reticulum (V) surrounding PC yolk vesicles (Y) and the egg membrane closely adhering to the oolemma. X 19,700.

B and C. Serial longitudinal sections near a constriction ring showing mitochondria associated with a multimembranous body (double arrows). Possible secretion of border spheres in 2 areas (single arrows). X 29,330.

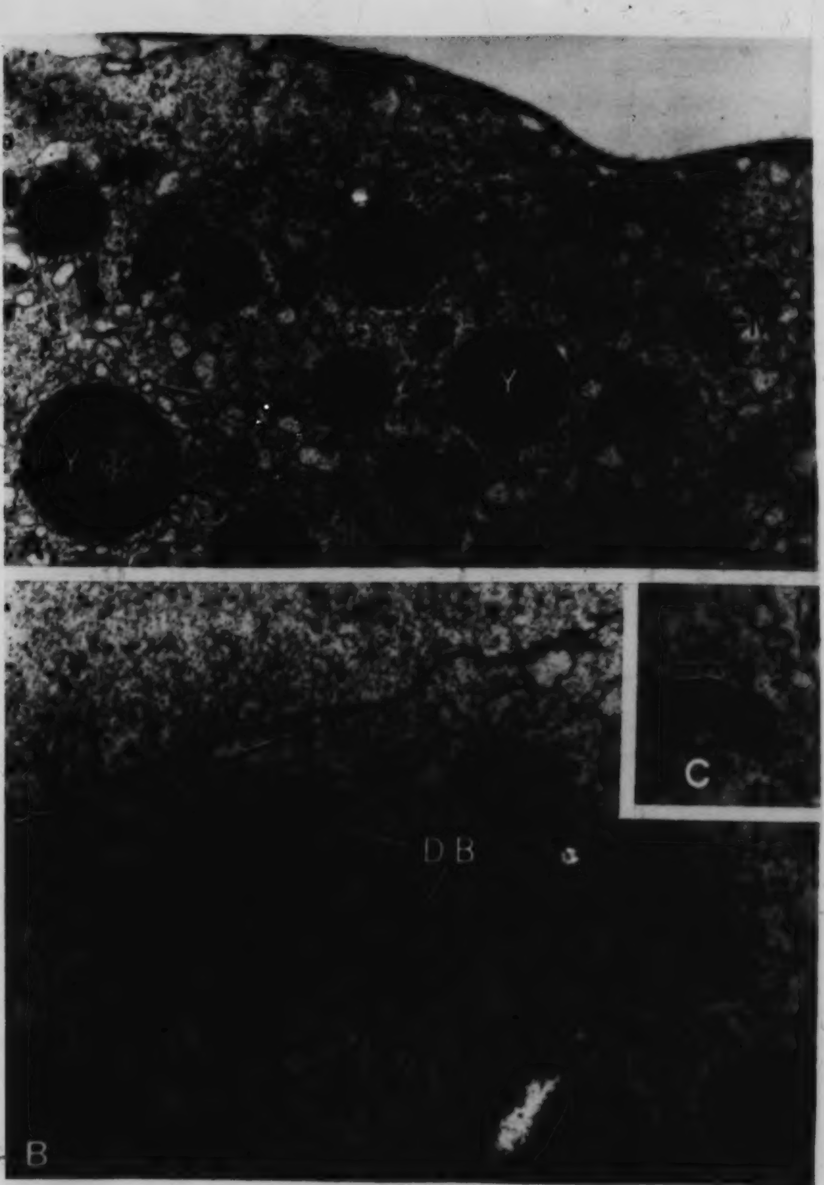

Y
Y Y
C
DB
B

Figure 44. *Pollicipes polymerus.*

A. Longitudinal section in the constriction ring area with dense bodies (DB), showing vesicularization of PC yolk (Y) as they are broken down. X 32,660.

B. Longitudinal section near a constriction ring. Note MVBs in various stages of release of vesicles into the cytoplasm. X 16,540.

Further yolk breakdown:

C. Vesicles internalized. X 20,400

D. Formation of a double membrane surrounding vesicles. X 20,400.

E. Most of the internal material is released. X 20,400.

F. Multivesiculate body is associated with glycogen. X 40,000.

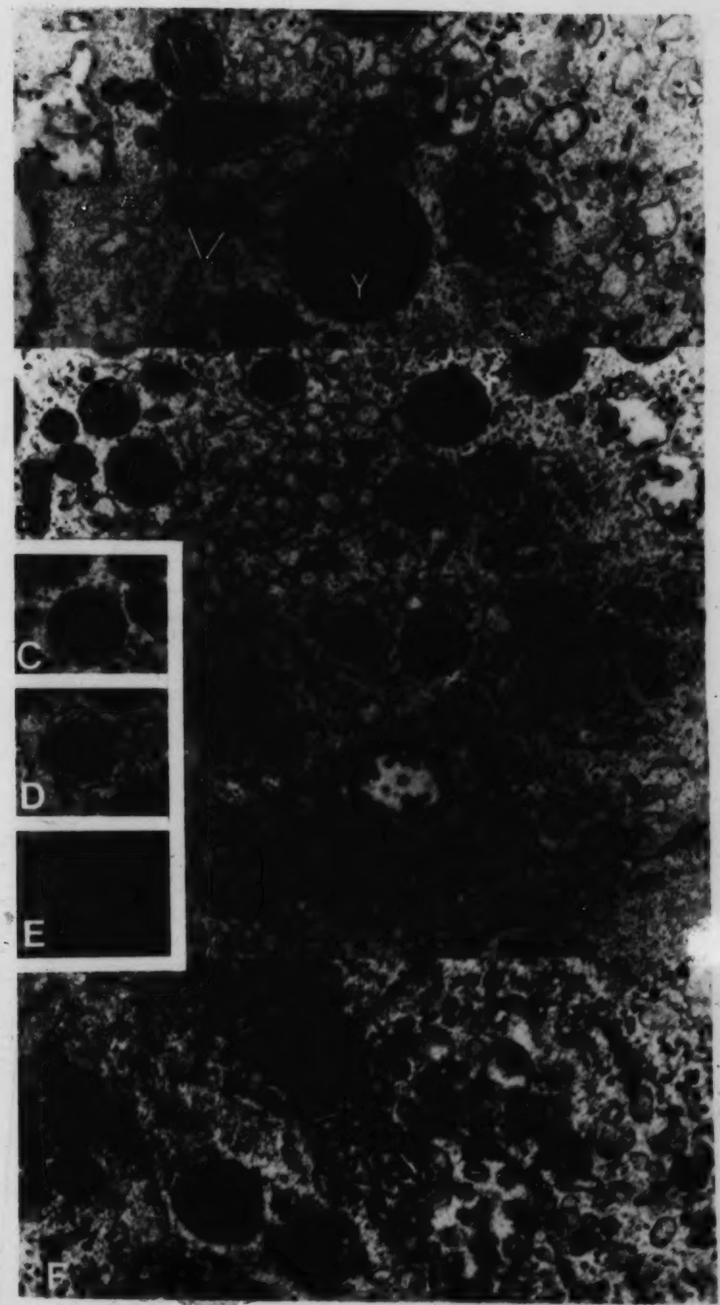
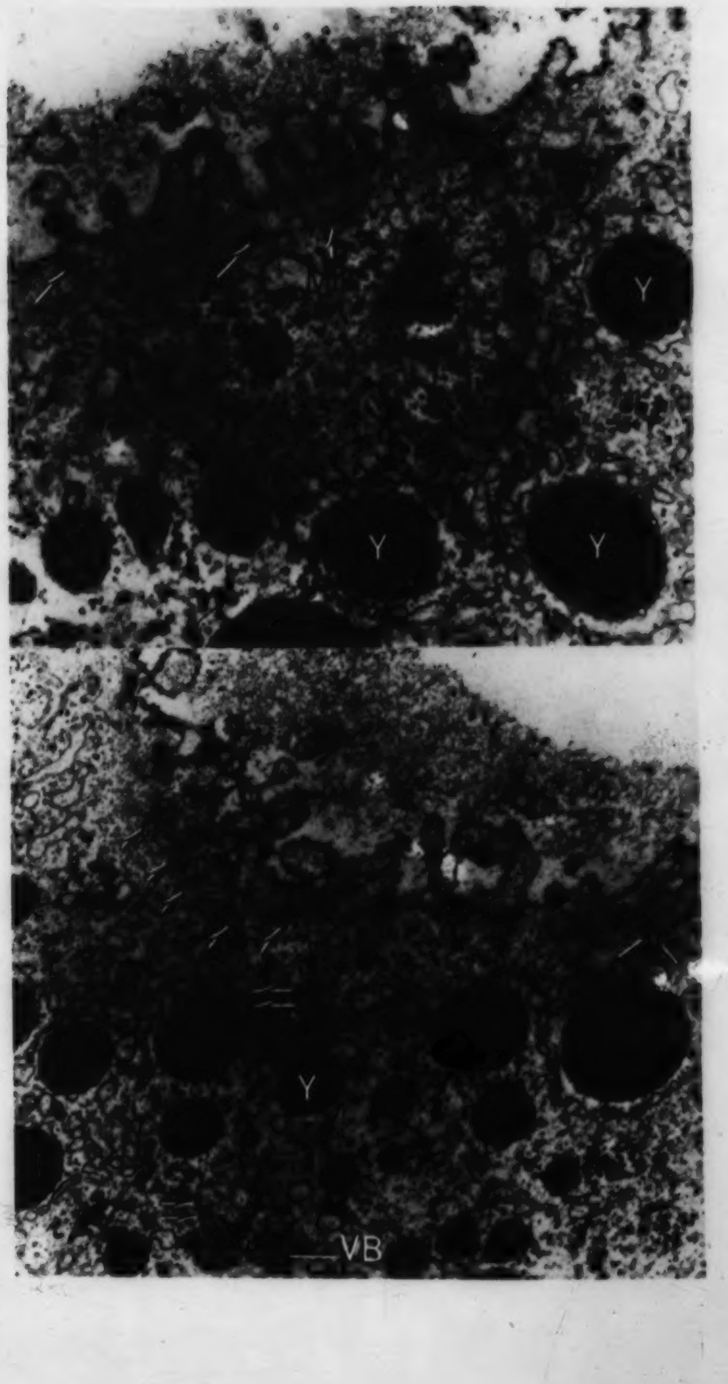

V
Y
B
C
D
E
F

Figure 45. *Pollicipes polymerus.*

A. Longitudinal section immediately vegetal to a constriction ring, showing vesicles with enclosed material (arrows), wisps of microfilaments (MF) and smooth vesicles surrounding yolk (Y). X 21,810.

B. Longitudinal section of a vegetal constriction ring, showing vesicular endoplasmic reticulum (V) surrounding yolk, MVBs breaking up (VB) and chains of membrane-bound dense material apparently emanating from the MVBs (double arrows), dense zones of microfilaments (single arrows) and intact yolk (Y). X 16,540.


Y
Y
Y
Y
VB

vegetal-position constriction ring (Figs. 46A to C). The animal pole without constriction is convex, a constriction in the middle of the egg is barely concave, and a vegetal constriction is acutely concave.

The egg membrane adheres to the oolemma at the animal pole (Fig. 46A). It begins lifting midway down the egg (Fig. 46B), and is lifted up to 14 μ in length and 3 μ in height away from the oolemma in vegetal constriction rings (Fig. 46C).

Microvilli occur only at constriction zones near the vegetal pole. Differences are also observed in the cortex of the 3 areas at higher magnification. Glycogen granules and organelles such as smooth endoplasmic vesicles extend to the oolemma at the animal pole (Fig. 46A). A shallow cortex of apparent homogeneous density is observed midway down the egg in a constriction ring (Fig. 46B). However, the cortex may extend to 6 μ in width and 0.5 μ in thickness in the most constricted part of a vegetal ring, as well as just vegetal to it and 0.25 μ in thickness just animal to it (Figs. 46C and 47). The ring width coincides precisely with the concave portion of the cell's profile, i.e., between inflection points in its outline (Fig. 46C, asterisks).

A circumferential band of dense cortical material is observed in equatorial sections of a vegetal ring (Fig. 48B). The overlying plasma membrane erupts into numerous microvilli in the constriction ring, but lacks microvilli and has no underlying differentiated cortex in regions outside the immediate constriction ring (Fig. 48A). The width of the constriction ring cortex is about 10 to 15 times greater than that of the non-constriction ring cortex (compare Figs. 48A and B). The egg membrane has also lifted off a great distance from the oolemma in the constriction ring plane. While organelles are found in the cortex at

Figure 46. *Pollicipes polymerus.*

A. Longitudinal section of the animal pole, showing a convex outline. The egg membrane adheres to the oolemma. X 16,760.

B. Longitudinal section of a middle-position constriction ring. The egg membrane has not yet lifted. Note the slightly concave outline, the thin cortex, and small microvillous protrusions. X 10,800.

C. Longitudinal section of a vegetal constriction ring. The egg membrane is elevated above the constriction. Note the dense streaks (arrows) running into the constriction ring and the deeply concave outline between asterisks. X 4,860.

Figure 46. *Pollicipes polymerus.*

A. Longitudinal section of the animal pole, showing a convex outline. The egg membrane adheres to the oolemma. X 16,760.

B. Longitudinal section of a middle-position constriction ring. The egg membrane has not yet lifted. Note the slightly concave outline, the thin cortex, and small microvillous protrusions. X 10,800.

C. Longitudinal section of a vegetal constriction ring. The egg membrane is elevated above the constriction. Note the dense streaks (arrows) running into the constriction ring and the deeply concave outline between asterisks. X 4,860.

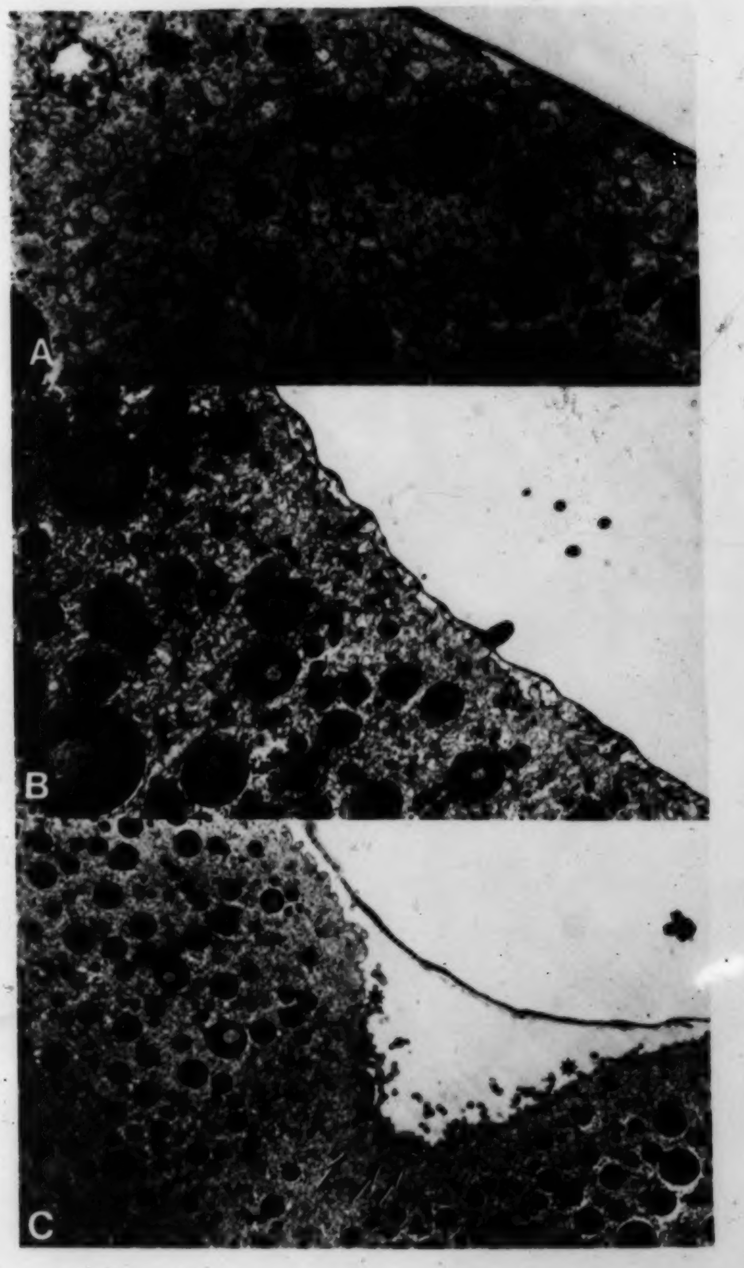

A
B
C

Figure 47. *Pollicipes polymerus*. Grazing longitudinal section of a vegetal constriction ring showing dense streaks running into the plane of the ring and a series of MVBs (dense-1 to light-5) in the interior of the egg. The cortical streaks are not homogeneous; some are denser (arrows) than others. The animal (A) and vegetal (V) poles are indicated. X 11,710.

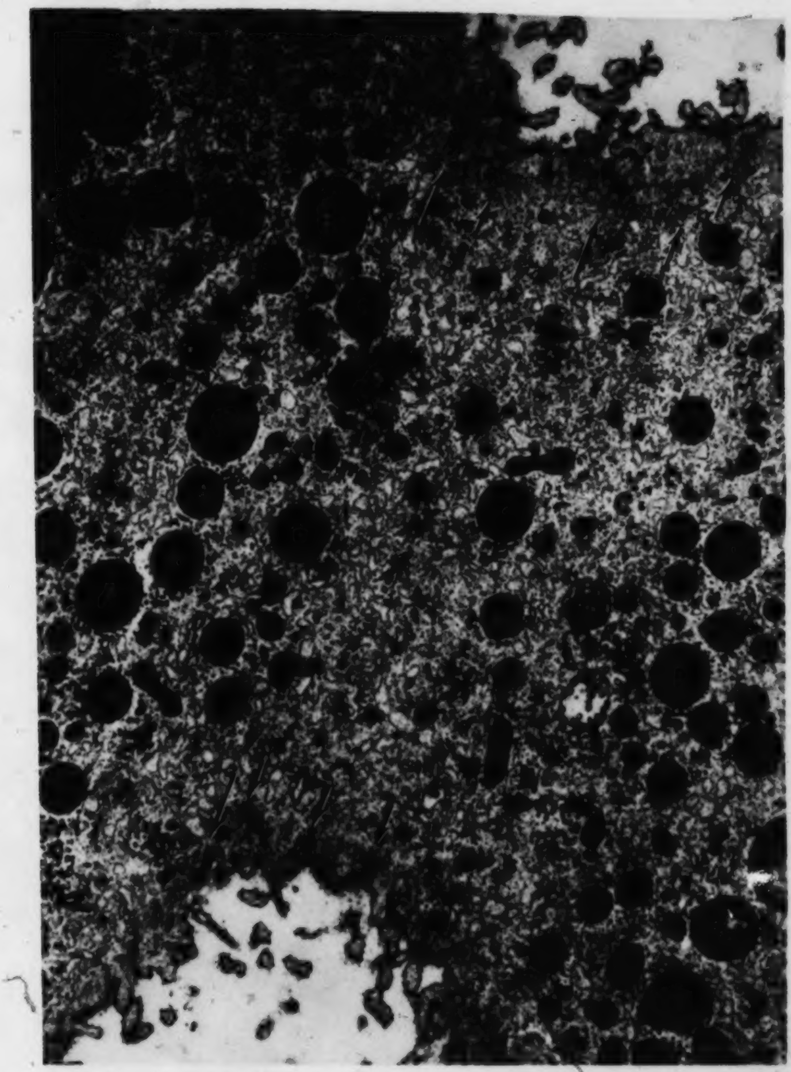
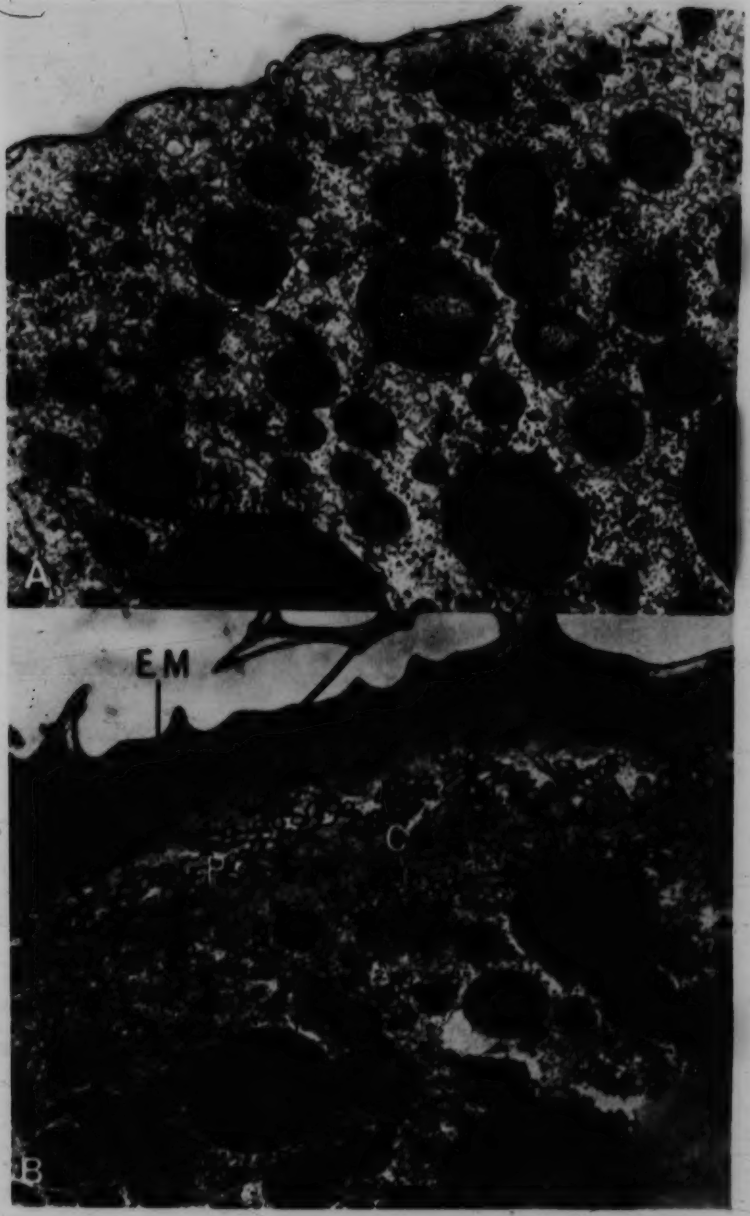


Figure 48. *Pollicipes polymerus.*

A. Equatorial section of an egg between constriction rings. Note the thin cortex (C) (less than 0.1 μ) and organelles close to the oolemma. X 9,030.

B. Equatorial section of a vegetal constriction ring. Note the thick cortex (C), numerous microvillous protrusions (P) and lifted egg membrane (EM). X 16,910.


A
EM
C
P
B

points other than constriction zones, there is an absence of organelles in the constriction zone cortex itself (Figs. 46A to C). Some material presumably excludes these organelles from the cortical zone. Thin microfilaments (45 to 60 Å diameter) are observed arranged in a circumferential meshwork in the cortex, apparently only at constriction ring zones.

Dense bundles of filaments radiate in streaks from the oolemma in grazing longitudinal section (arrows, Fig. 47). Although bundles of filaments are discerned, they are obliquely sectioned, and in some cases a meshwork of filaments is observed beneath the circumferential bundles (Figs. 49A and B). The apparent absence of longitudinal sections of long microfilament bundles may be due to the fact that they primarily have a meshwork arrangement.

Filaments extend into microvillous protrusions (Fig. 49A), and although most filaments tend to group in meshworks just below the oolemma, some filaments may insert or adhere to the oolemma (Fig. 49B). When grazing longitudinal sections are taken at the vegetal tip of the egg, such that the vegetal lobe formed from the constriction ring has not yet been cut (Fig. 37D), some filament bundles are observed oriented in a paraequatorial fashion (circumferential above or below the cell's equator) (Figs. 50A to C). Likewise, wisps of circumferentially arranged filaments are observed adjacent to filament meshworks in equatorial sections (Fig. 51A). However, only meshworks are found in the middle or toward the animal half of the egg in constriction rings, and as one cuts toward the vegetal pole, the paraequatorially oriented filaments appear.

Figure 49. *Pollicipes polymerus.*

A. Longitudinal section of a vegetal constriction ring, showing a filamentous meshwork in the constriction ring (arrows) and filaments (F) in the microvillous protrusions. X 69,160.

B. Longitudinal section of a vegetal constriction ring, showing possible filament insertions into the oolemma (arrows). X 56,100.

F
A
B

Figure 50. *Pollicipes polymerus.*

A to C. Grazing longitudinal sections of vegetal constriction rings. Filaments are oriented equatorially (arrows). A. X 28,000. B. X 29,520. C. X 71,140.

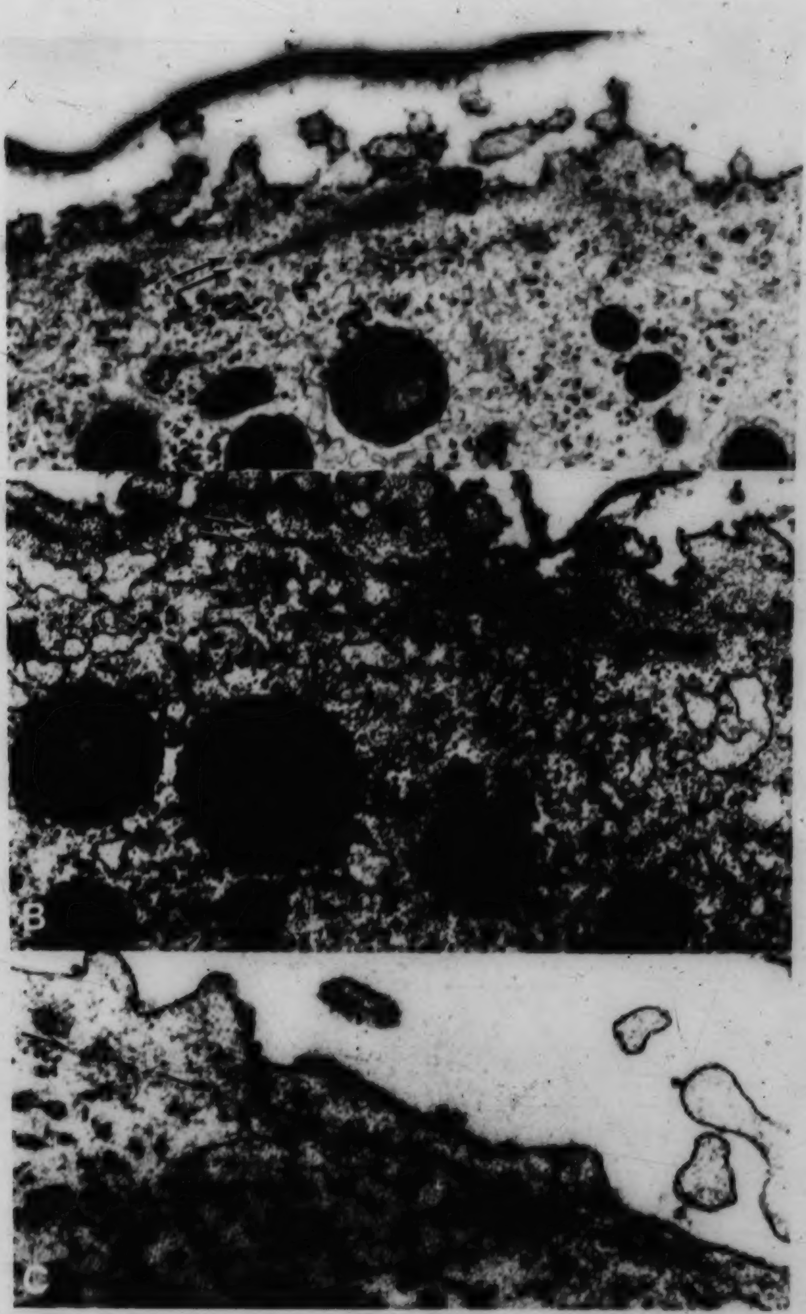
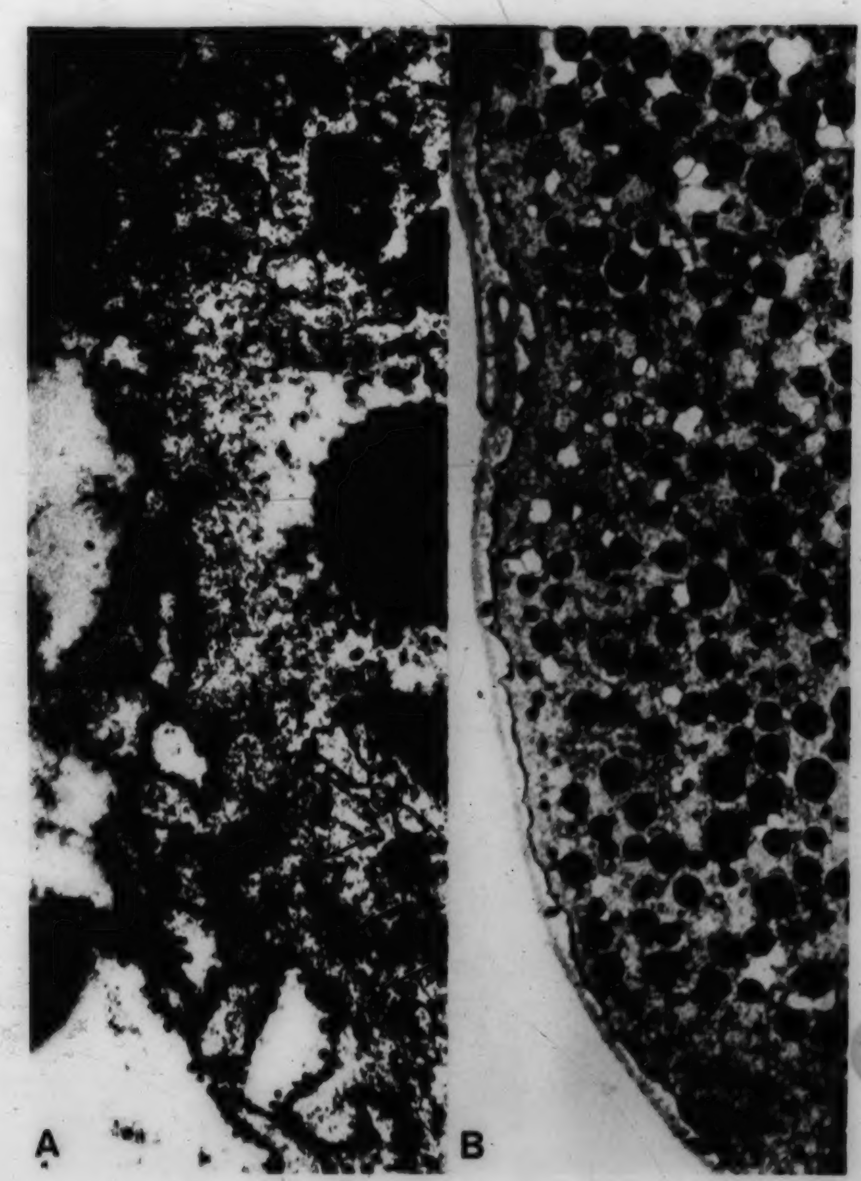

A
B
C

Figure 51. *Pollicipes polymerus.*

A. Equatorial section of a vegetal constriction ring, showing wisps of equatorially arranged filaments (arrows). X 55,610.

B. Longitudinal section of an egg treated with 20 μg/cm^3 CCB for 5 min and briefly washed in sea water. A middle-position constriction ring is partly dampened (1), and a vegetal ring is wholly dampened (2). X 5,450.


A
B

The arrangement of microfilaments in the cortex of a vegetal constriction ring shows variations along the animal-vegetal axis: 1) animal to the deepest part of the ring, 2) deepest part of the ring, and 3) vegetal to the deepest part of the ring. In the "animal" area, most filaments are parallel to the oolemma or in meshworks, while in the base of the ring and slightly vegetal to it, many filaments are equatorially arranged (running in the plane of the ring). Moving vegetally a bit more, there are only wisps of unarranged filaments.

A number of small, membrane-bound spheres were observed in the cytoplasm of the constriction ring. They are likely formed from the MVBs and secreted outside the oolemma via reversed micropinocytosis (Figs. 45A and B). Occasionally they were seen adhering to one another and forming short chains (Fig. 45B). Further, Szollosi (1970) suggested that an exocytosis of a flocculent to filamentous material designated as mucoid may accompany furrowing in cleaving eggs.

When the average number of various organelles and other structures found in constriction ring and non-constriction ring areas are compared statistically, microvilli, mitochondria, MVBs, and glycogen granules differ significantly in their dispersal pattern (Table 21), i.e., these organelles are not evenly distributed, they are found significantly more often in constriction rings.

Effects of Cytochalasin B

The immediate effect of CCB observed at the light microscopic level is cessation of peristaltic movement from the animal to the vegetal pole and a gradual dampening of the amplitude of constriction rings, themselves. CCB effects were reversible in cells treated with up to

Table 21. *Comparison of the distribution of some organelles in* Pollicipes polymerus *eggs by means of a* t-*test. (No. of eggs are in parentheses.) NS: not significant;* * $P < 0.05$; ** $P < 0.001$; *SE: standard error.*

	In plane of constriction ring				Not in plane of constriction ring				Level of significance
		Mean no. of organelles		S.E.		Mean no. of organelles		S.E.	
Protein Yolk	(14)	21.4	±	5.0	(13)	13.0	±	2.0	NS
Dense Bodies	(14)	10.5	±	2.9	(13)	2.6	±	0.9	*
Glycogen granule aggregations	(14)	25.9	±	6.5	(13)	8.5	±	1.5	*
Multivesiculate bodies	(14)	3.3	±	0.8	(13)	1.0	±	0.3	*
Mitochondria	(14)	12.7	±	2.5	(13)	3.9	±	0.9	*
Microvilli	(14)	22.4	±	3.4	(13)	0.6	±	0.2	**

1 μg/cm^3. One also observes the lack of constriction rings at the ultrastructural level (Figs. 51B and 52C). Eggs treated with 0.5 μg/cm^3 CCB evidenced vacuolation, as well as adhesion of the egg membrane to the oolemma (Figs. 52C and E). In place of concavities where the rings should be, the cell is generally convex. Almost no cortical blebbing is observed as in some other studies of CCB-treated eggs (Schroeder, 1970b).

The salient features of an egg treated with 20 μg/cm^3 CCB for a shorter period are a partly dampened middle-position constriction ring and a wholly dampened vegetal ring (Fig. 51B). In the cortex of a middle-position ring, patches of dense material including cross sectional and oblique views of 45 to 60 Å microfilaments are found in remnants of microvilli (Fig. 52A). In the vegetal cortex where one would expect to find signs of the constriction ring if it were present, there are instead dense patches of amorphous material (Fig. 52B). Whether these patches represent parts of a constriction ring which failed to assemble properly, remnants of one that was dissociated, or merely cytoplasmic condensation is unknown. However, differentiation in the form of a defined constriction ring is apparently inhibited by the action of CCB, as noted for other filament networks (Yamada and Wessells, 1973). Only a few microvilli which are normally found in the constriction ring remain.

Other effects of CCB on the fine structure of fertilized *P. polymerus* eggs are an increase in numbers of vacuoles, most being associated with PC yolk or organelles (Fig. 52A), some compression or contraction of mitochondrial cristae, a redistribution of glycogen granules from aggregations to a more heterogeneous arrangement, and the formation of more myelin bodies than is normally seen (Fig. 52D). It is significant

Figure 52. *Pollicipes polymerus.*

A. Higher magnification of the middle-position constriction ring (Fig. 51B, area 1). Vacuoles (V), patches of amorphous dense cortical material (asterisks), and remnants of microvillous protrusions (P) are visible. X 33,640.

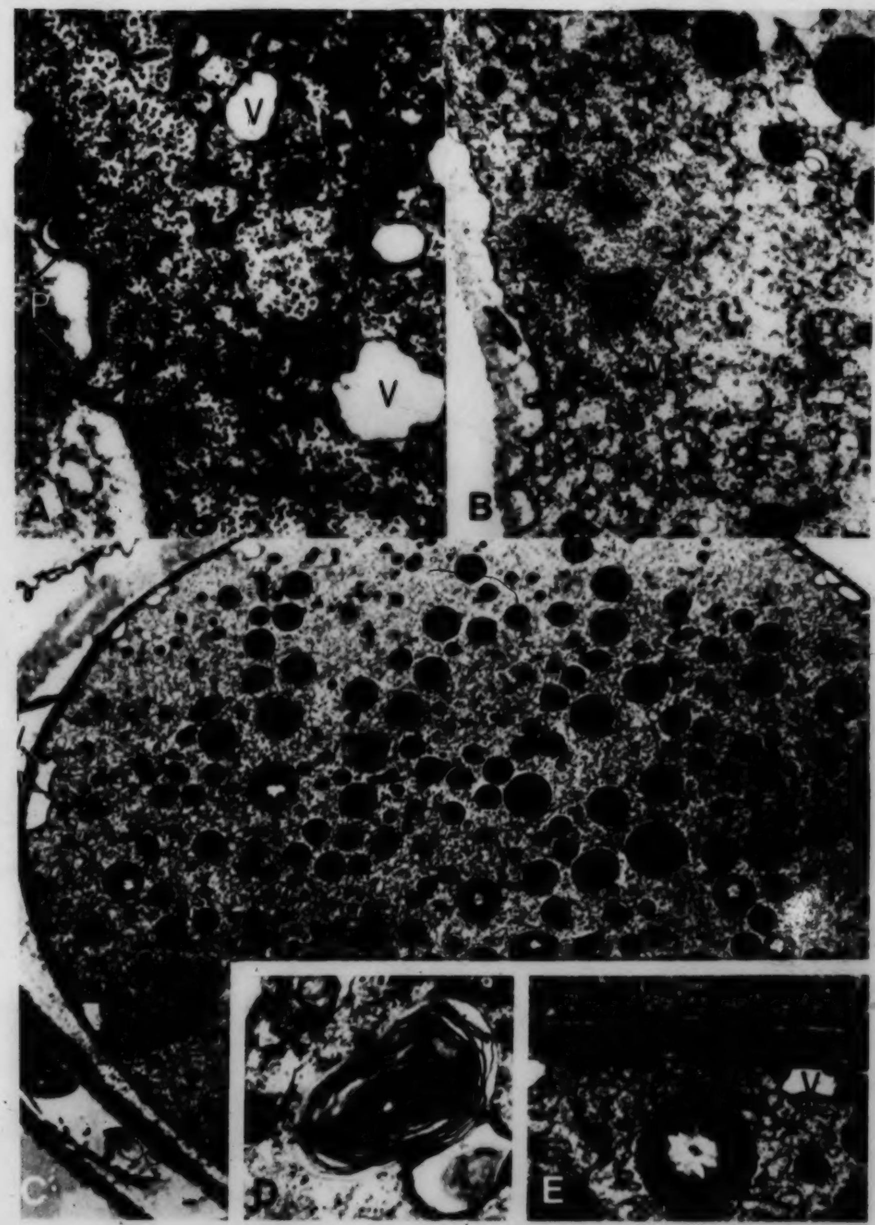
B. Higher magnification of a vegetal constriction ring (Fig. 51B, area 2). Dense patches of amorphous cortical material (asterisks), microtubules (MT) and a few microvillous protrusions are visible. X 10,550.

C to E. Longitudinal section of an egg in an ovigerous lamella treated with 0.5 μg/cm^3 CCB for 11 min and washed briefly.

C. There are no constriction rings, but there are several areas where vacuolation (V) is occurring. X 4,750.

D. Myelin body. X 25,200.

E. Vacuoles (V) forming and/or fusing. X 12,760.


V
P
V
V
A
B
C
D
V
E

to note that CCB has no marked effect on the structure of microtubules (Fig. 52B).

Effects of the Dimethylsulfoxide Control (DMSO)

CCB was dissolved in up to 0.1% DMSO. No cessation of the peristalsis was observed in eggs treated in 0.1% DMSO for 15 min. An equatorial section through a vegetal constriction ring of a DMSO-treated egg exhibits 60 Å microfilaments oriented equatorially (Fig. 53A). The alteration of cytoplasmic particle distribution in CCB-treated eggs has been observed in other DMSO-treated cells (Schroeder, 1970b).

Effects of Antimycin A

No disruption of mitochondrial cristae is observed in ethanol-treated controls (Fig. 53C), although there is a slight disruption of cytoplasmic particles and the egg membrane. Most mitochondria in antimycin A-treated eggs exhibit inclusions and/or disrupted cristae (Fig. 53B).

Discussion

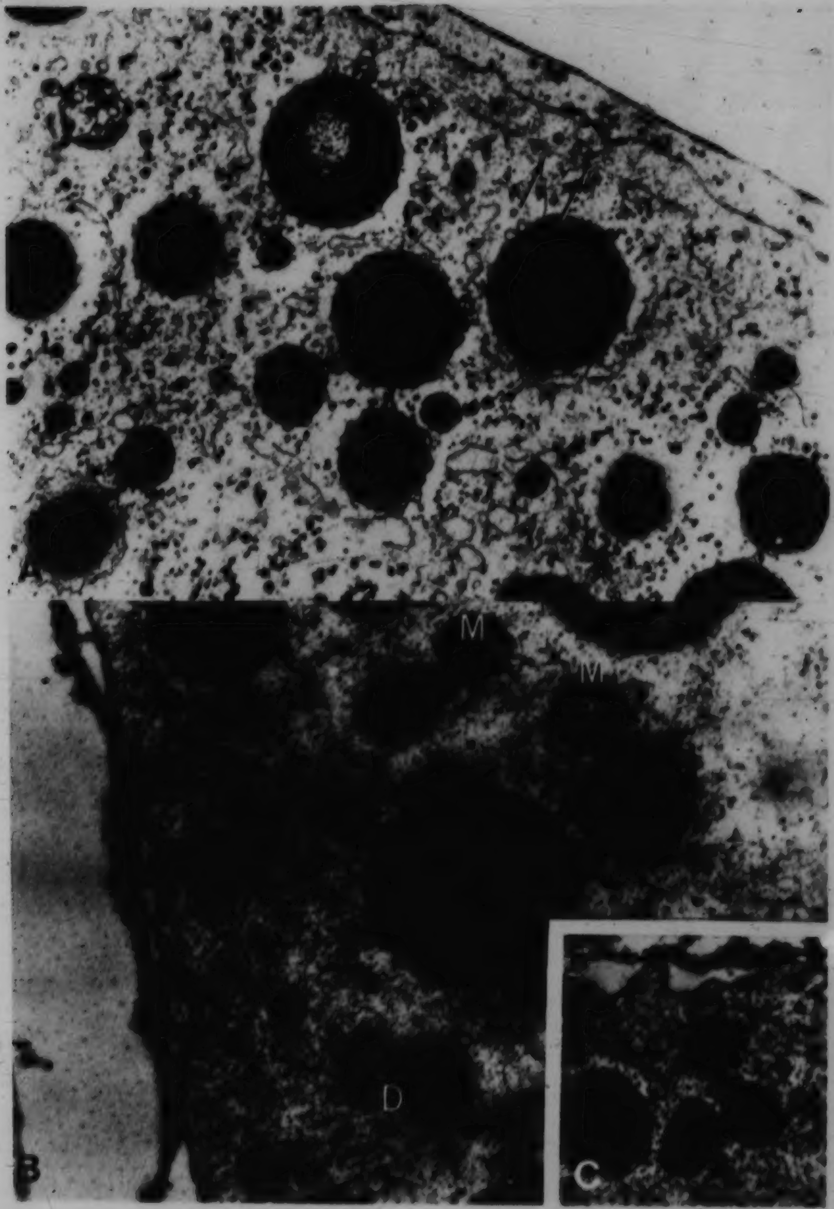
The fertilization membrane may be formed from the only other membrane known to exist previous to its appearance, the vitelline membrane (Woods, 1969). According to Woods (1969), the vitelline membrane consists of a dense, fibrous material 2 μ thick. A flocculent or fibrous material is found lying between the fertilization membrane and the oolemma (Figs. 35C, 36C and D); this may have originated in the ovarial lumen and moved through vitelline membrane channels (Woods, 1969), or it may be a remnant from the fertilization membrane reaction, since there exists a difference of about 1.85 μ in thickness between the vitelline and fertilization membranes.

Figure 53. *Pollicipes polymerus.*

A. Equatorial view of a vegetal constriction ring showing equatorially arranged microfilaments (arrows). The egg was treated with 0.1% dimethylsulfoxide for 15 min (control). X 24,820.

B. Section of an egg treated with antimycin A (500 $\mu g/cm^3$) for 90 min. Note mitochondrial disruption (M) and dense inclusions in mitochondria (D). X 29,050.

C. 1% ethanol control showing normal mitochondria. X 21,810.


M
M
D
B
C

Experimental evidence indicates that the peristaltic constrictions in this barnacle egg are probably caused by microfilaments not by microtubules. Protein synthesis may be necessary for the constrictions, but oxidative phosphorylation is not.

Transmission electron microscopy has disclosed that thin microfilaments are arrayed in a cortical meshwork, as well as paraequatorially in the vegetal part of the egg, in peristaltic constriction rings of *P. polymerus* eggs. However, a complete annulus of interdigitating filaments was not found in the ring. Contractile rings consisting of thin microfilaments arrayed circumferentially have been identified in cleaving HeLa cells (Schroeder, 1970b) and in eggs of *Stomatoca atra* (Schroeder, 1968), *Arbacia punctulata* (Schroeder, 1969; Tilney and Marsland, 1969; Goodenough *et al.*, 1968), *Loligo pealii* (Arnold, 1968, 1969, 1971), coelenterates (Szollosi, 1968, 1970), *Ambystoma mexicanum* (Bluemink, 1970) and *Xenopus laevis* (Bluemink, 1971a,b). In each case, microfilaments of the same size range as in the *P. polymerus* egg were described. In analogy to the above findings, the microfilaments in *P. polymerus* eggs are thought to generate the force of constriction.

A 3 dimensional meshwork of thin filaments anchored to the oolemma may be better adapted for generating a constriction ring moving in 2 planes simultaneously (inwards and along the long axis of the egg) than simply an annulus of filaments as in the relatively static rings of cleaving cells. It is likely that the equatorial bundles of filaments may be interconnected with the cortical network of filaments and that these interconnections may function as anchors during constriction at the particular point along the egg's axis. Filaments were found extending into the microvilli in most longitudinal sections, which also suggests an anchoring function, although filaments may also assist in microvillous extension (Tilney and Cardell, 1970). Bluemink (1970) observed that in the furrow of *Ambystoma* eggs, the bottom of the groove is always thrown into folds and the filaments are not always equally distributed over the area of contraction, thus suggesting that the filaments contract harmoniously in bundles which are not necessarily continuous. This may also be the case in *Pollicipes* eggs in which discrete groups of filaments were observed. It does not seem unusual, then, that the rate of constriction movement decreases while the rings deepen at the vegetal pole (Lewis *et al.*, 1973), since more filaments must polymerize and orient here than towards the animal pole.

Szollosi (1970) felt that cytoplasmic membrane-bound granules (100 to 300 mμ diameter) with tightly-packed filamentous contents were secreted to create extracellular filamentous material adjacent to the base of first cleavage furrow invaginations similar to the extracellular filaments seen in constrictions of *Pollicipes*.

Based on the morphological evidence, an hypothesis of the mechanism of peristaltic constriction is proposed. In order to accurately model the forces which resist the constriction, one must know the physical characteristics of both the membrane and the cytoplasm. These might reasonably be modeled as an elastic solid and an incompressible viscous fluid, respectively. Although this is beyond the scope of this research, some qualitative conclusions can be drawn from an understanding of fluid mechanics. First, it should be noted that several types of forces (e.g., viscosity, surface tension, hydrostatic pressure, and inertia) could potentially be important in determining the dynamics of the peristaltic constrictions. However, previous studies of cell movement

(Allen, 1974; Hancock, 1953; Hiramoto, 1958) indicate that viscosity and surface tension forces are predominant. Considering this and the non-Newtonian[9] nature of cytoplasm (Allen, 1974), makes the understanding of peristaltic motion very difficult.

Consideration may be given to 3 possible arrangements of microfilaments that could theoretically cause constrictions: microfilaments radiate from the central axis of the egg (Fig. 54A); they form rings at or just below the surface of the egg (Fig. 54B); and they run in a criss-cross manner resembling a braided rope (Fig. 54C). The spoke arrangement is clearly inefficient, since filaments would interfere with any movement of the cytoplasm and organelles along the egg which is known to occur at this time. The ring arrangement does not have this problem. However, the filaments would not provide any strength to the membrane or cortex parallel to the long axis of the egg which seems necessary, since the membrane is probably stretched or grows in that direction at or near the constriction (Fig. 55B).

The arrangement of filaments in Figures 54C or D takes both of these problems into account. Further, the 2 sets of filaments running diagonally may slide between each other, giving the desired motion. The diagonal angle could be adjusted to account for the different amounts of strength necessary in the meridional and equatorial directions.

The microfilaments in vegetal constriction rings appear to be oriented as in Fig. 54D and those in the animal constriction rings are oriented as in Fig. 54C, based upon the evidence of hundreds of micrographs studied. In addition, when the egg membrane is torn (Fig. 37A), the

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[9] In a Newtonian fluid shear stress is linearly proportional to the rate of shear strain.

Figure 54. *Pollicipes polymerus*. Perspective view of eggs sliced in constriction rings near the vegetal pole (top), showing 4 possible arrangements of microfilaments (A to D). Diagrams C and D are considered to accurately model actual filament arrangements.

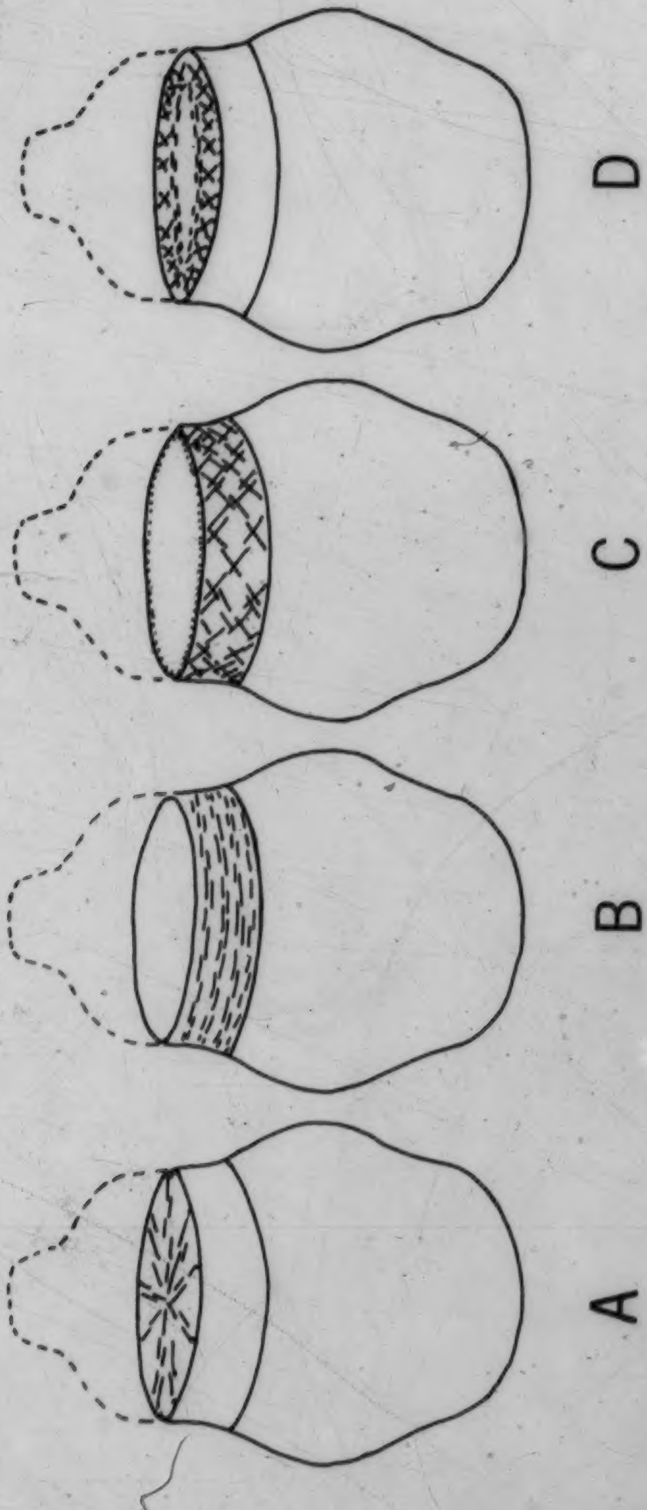
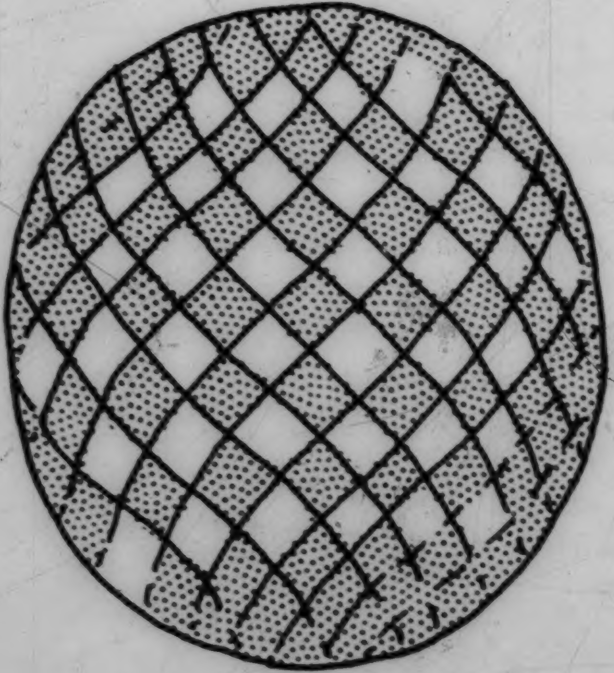
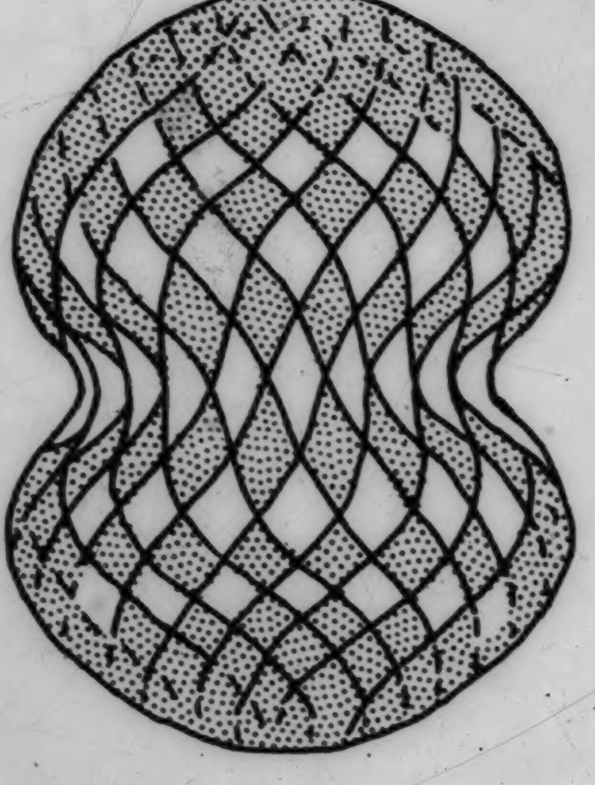

D
C
B
A

Figure 55. *Pollicipes polymerus.* Perspective diagram of a spherical egg (A) with diagonals drawn from a regular grid placed around it (parallel equidistant equatorial lines plus equidistant meridional lines which converge at the poles). The egg has a single constriction ring in B. If a central square in A is observed in B, it has elongated in the long axis of the egg and shortened in the equatorial axis, forming a diamond. Since direct measurements were not made of changes in the oolemma during peristaltic constriction, it is not known if this model accurately represents membrane deformation, but it is probable that constriction could produce such a deformation.



A



B

constriction rings pull in tighter, and the egg elongates about 1 1/2 times its normal length.

If one regards filament orientation relative to position within the vegetal constriction ring, just animal to the ring (where the ring has just passed), filaments are in meshworks or even perpendicular to the ring, while filaments in the deepest part of the ring and just vegetal to it (constriction is moving into this area) are mostly parallel to the plane of constriction with an attached meshwork (Figs. 47 and 49A). The cortex just in front of the ring may have been stimulated in some manner such that precise filament orientation is accomplished from wisps of filaments ahead of the advancing ring. In the cortex following ring formation, filaments become disoriented in relation to the plane of constriction. The filaments are likely depolymerizing to some precursor form, since they are only detected in the constriction ring cortex (the constriction ring is a transitory organelle) and as has been previously observed (Bluemink, 1970; Cloney, 1966), unaligned filaments are found before contraction. Thus, previously non-oriented filaments may polarize and aggregate (Goldman *et al.*, 1973; Schroeder, 1973b), suggesting that orientation is more closely linked to contraction than to assembly.

Arnold (1969) implied that the larger dense bodies (150 to 300 mμ) in *Loligo*'s cleavage furrow released 45 Å filaments into the furrow, but no evidence of this was found in the *Pollicipes* egg.

Although organized filament arrays may not be necessary for contraction, and since this function is apparently capable of being performed by a filament network (Yamada *et al.*, 1970, 1971) as in constriction rings in the animal portion of the *Pollicipes* egg, it has been suggested that they may serve to augment motive force when more powerful local movement is required (Komnick *et al.*, 1973) as in the vegetal portion of the egg.

It is known that: 1) filaments are present at the sites in eggs where they could cause shape changes by contractile activity; 2) alteration in morphology of the filament system with CCB inhibits the shape changes and 3) both the filament system and CCB-sensitivity are apparently transient aspects of morphogenesis.

Although one must be very cautious in assuming that because filaments are present in a contractile cell, the filaments must be the motile force, in the case of newly-fertilized *P. polymerus* eggs, it is difficult to conceive of a function other than contraction, as no other organelles (microtubules, in particular) are apparent which could be responsible for the movement. And although it is conceivable that forces moving cell margins might originate in contractile elements in the plasma membrane, knowledge of the ultrastructure and properties of biological gels (Frey-Wyssling, 1953) suggests that filament networks, if passive, would give rise to highly viscous cytoplasm, thus promoting much resistance. There is also no evidence for contractile membrane proteins or lipids. Therefore, it is more likely that the filaments are active in force-generation. In addition, thin filaments were observed in the cleavage furrow during first polar body formation and in a dense layer in the second polar body of *Pollicipes*. Although no cells were studied during cleavage of blastomeres, this observation of filaments during polar body formation lends credence to the theory that filaments are involved in contractile activity in the *Pollicipes* egg. Decoration of thin microfilaments with heavy meromyosin (HMM) establishes their actin-like behavior as in cleaving cells (Schroeder, 1973a) and biochemical demonstration of actin and/or myosin elements is necessary for conclusive proof of filament contractility in the barnacle egg contractions.

During the last 10 years an extensive literature has accumulated on studies of contractile phenomena and their possible relationship with actin-like, thin microfilaments (for reviews see Jahn and Bovee, 1969; Wessells *et al.*, 1971; Allison, 1973; Wessells *et al.*, 1973; Durham, 1974; Pollard and Weihing, 1974; Yamada, 1974). It has been suggested that the ultrastructural patterns of filaments observed actually reflect their biochemical organization: 1) parallel filaments represent highly organized actin, 2) networks are less organized and 3) faintly fibrillar or amorphous masses reflect low concentrations and/or little organization (Yamada, 1974).

Microfilament networks, always in a subplasmalemmal region, have been observed in: undulating membranes of motile cells (Buckley and Porter, 1967; Goldman and Follett, 1969; Spooner *et al.*, 1971, 1973; Axline and Reaven, 1974; Buckley, 1974; Malech and Lentz, 1974), extension of nerve cell axons (Yamada *et al.*, 1970, 1971), wound closure (Bluemink, 1972), endocytosis (Singh, 1974), and phagocytosis (Reaven and Axline, 1973). The latticework is thought to have multiple insertion points on the inner face of the membrane (Wessells *et al.*, 1973), producing active shearing forces comparable to those of the sliding filaments in muscle (Burnside, 1971, 1973; Huxley, 1973; Schroeder, 1973b; Pollard and Weihing, 1974). Careful measurements of contractile ring volume during cleavage by Schroeder (1972) suggest that volume decreases during the second half of cleavage and a contraction-related disassembly of contractile ring filaments occurs, at least during the first part of contraction. The constriction rings vary in thickness from the leading edge to the deepest part to the trailing edge in *P. polymerus* eggs, which is analogous to stages of cytokinesis; therefore, a contraction-related assembly/disassembly of filaments may also be important here.

Microtubules were not often observed in the constricting egg, and when noted, persisted after CCB treatment. It can be concluded from morphological evidence that microtubules probably play no active role in the constriction ring movement. Since microtubules are not found in great numbers in elongating *P. polymerus* eggs, they may not be important in this function either, although they have been suggested as the "agents of cell elongation" in other systems (Byers and Porter, 1964; Perry and Waddington, 1966; Arnold, 1967; Warren, 1968; Gibbons *et al.*, 1969; Schroeder, 1971).

CCB is a controversial drug in that its site of action may not be specific (for reviews see Holtzer and Sanger, 1972; Sanger and Holtzer, 1972; Pollard and Weihing, 1974; also Bluemink, 1971; Mizel and Wilson, 1972). Yamada and Wessells (1973) showed that the CCB effect was not due to inhibition of glucose uptake in glial and nerve cells, and it seems unlikely that transport of extracellular nutritive substances is necessary in fertilized *P. polymerus* eggs which have been grown *in vitro* to hatching (Lewis, 1975a,b). The masses of CCB-induced dense material is probably compacted aggregates of actin since similar CCB-induced material binds HMM in the cells which have been tested (Miranda *et al.*, 1974).

The CCB effect is reversible in *P. polymerus* eggs treated with 0.5 μg/cm^3 or less, but not when higher concentrations are applied

(Lewis *et al.*, 1973). Since the peristaltic constriction event is short-lived (4 to 6 h), it is possible that the time has passed when eggs will respond to filament activation; that is, constriction may only occur at a specifically-timed interval when the correct stimulus is present and CCB has not disrupted the microfilaments.

Vacuolation observed with CCB treatment may be created by the taking up of plasma membrane slack from the microvilli previously there, as suggested by Cloney (1966) and Wrenn and Wessells (1969). Williamson (1972) suggested that acute vacuolation may be induced by a variety of "unfavorable conditions" including certain inhibitors; therefore, this reaction to CCB may be due to a general effect rather than specific action on the membrane.

Organelles in the Peristaltic Constriction Ring Model

The relationship of the constriction ring microfilaments to other organelles was examined for possible clues as to the energy sources available for peristalsis. From Table 18 it is evident that mitochondria, glycogen granules and multivesiculate bodies are associated with the constrictions.

MVBs are commonly found in several animal eggs (Balinsky and Devis, 1963; Pasteels and DeHarven, 1963; Beams, 1964; Kessel, 1966; Norrevang, 1966, 1968; Schjeide *et al.*, 1966; Mekker and Zimmerman, 1970; Reverberi, 1970; Wischnitzer, 1970; Zissler and Sander, 1973; Dohmen and Verkonk, 1974; Kang, 1974) and are also associated with contractile events such as cleavage (Perry and Waddington, 1966; Arnold, 1968, 1969, 1971; Bluemink, 1970), but are not found in muscle cells. Production of yolk bodies in barnacle eggs (Woods, 1969; also tunicates, Kessel, 1966) is faintly reminiscent of MVB structure. However, there were no Golgi complexes and few ribosomes observed in constricting eggs which are necessary during vitellogenesis. While 1 type of PC yolk maturation consisted of membrane-bound precursor yolk granules first fusing centrally and later in the cortex of the yolk vesicle (Woods, 1969), the central portion of the yolk is always vesicular before the periphery in constricting *Pollicipes* eggs (Figs. 44A and C). Also, vesicles are observed first in yolk platelets as they break down. In addition, MVBs have long been suspected of lysosomal functions (Elliott, 1965; Conti and Klein-Szanto, 1973; Kang, 1974), including digestion of pinocytotic vesicles (Norrevang, 1966; Conti and Klein-Szanto, 1973), glycogen (Orci and Stauffacher, 1971), yolk (Pasteels and DeHarven, 1963; Norrevang, 1966; Bluemink, 1970; Zissler and Sander, 1973) and protein (Locke and Collins, 1967).

A lysosome the same size as adjacent yolk vesicles is shown in the actively contracting secondary mesenchyme cells of *Arbacia punctulata* gastrula (Tilney and Marsland, 1969). Its appearance is similar to the *P. polymerus* MVBs with vesicles embedded in a dark matrix. The polar lobe of *Bithynia* contains MVBs which are often ruptured, producing vesicles attached in strings (Dohman and Verdonk, 1974) as is found in constricting *P. polymerus* eggs (Fig. 45B). Crowell (1964) observed an association of ER vesicles with yolk in the polar lobe of *Ilyanassa* which suggested an active alteration of the yolk.

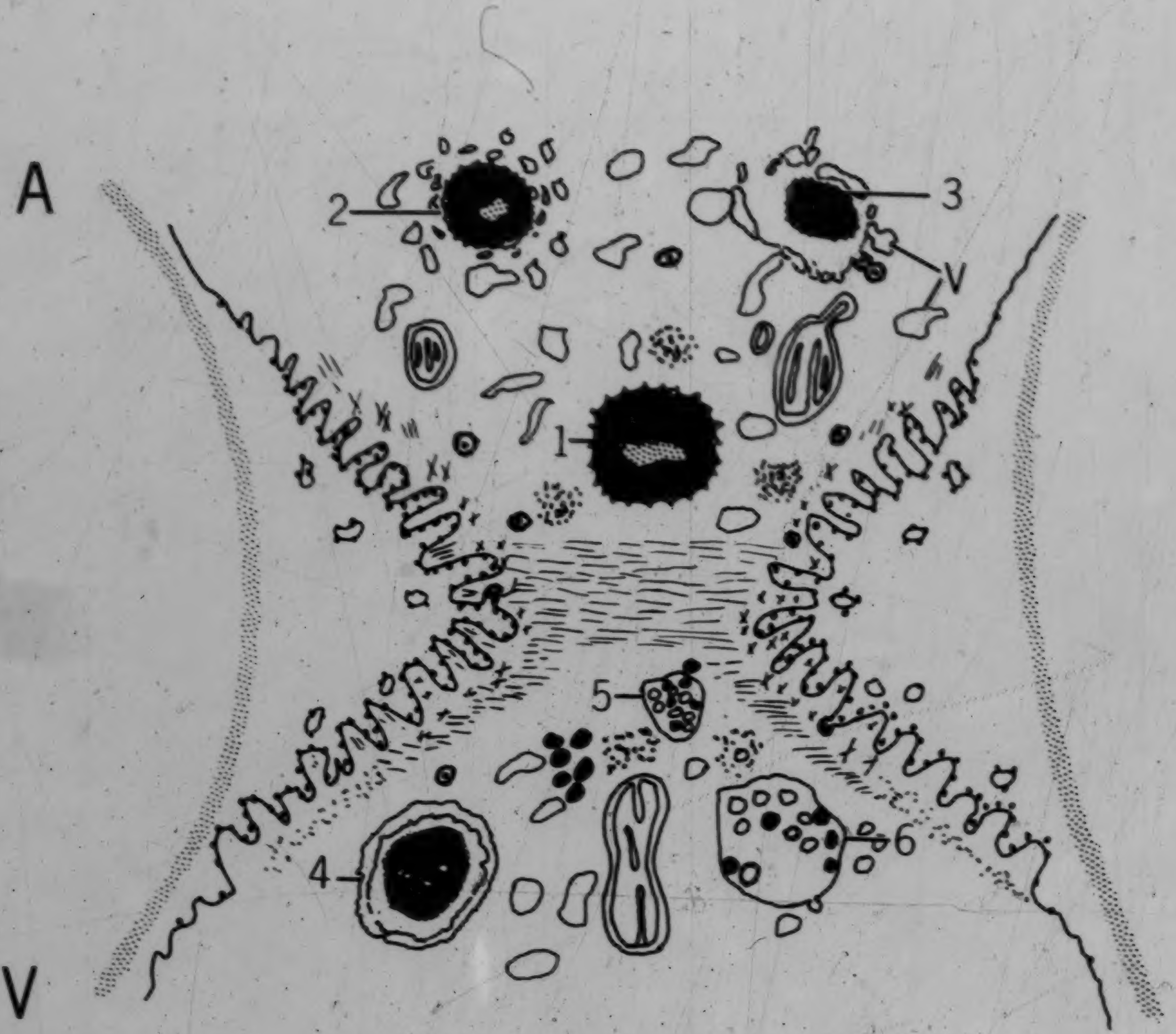
From evidence presented here, the MVBs of *P. polymerus* appear to transform in a continuous series of digesting PC yolk vesicles. According to Karasaki (1963) amphibian yolk is degraded for utilization by: 1) decreasing in thickness and disappearance of the superficial layer,

2) decomposition of the crystalline structure into units and 3) formation of laminar or vesicular membranes. In *P. polymerus* yolk a series of vesicular and laminar smooth ER vesicles form concentric to the yolk and fuse (Fig. 56), the central crystalline structure breaks down, and internal vesicles appear in the dark matrix. Finally, the vesicles appear to be released into the cytoplasm (see also Pasteels and DeHarven, 1963) and possibly some are secreted extracellularly. Bluemink (1970) also found MVBs involved with subcortical yolk granule degradation. Orci *et al.* (1972) suggested an important role for microfilament networks in secretion of certain endocrine cells.

Knobs of acid polysaccharide material similar in morphology to the border spheres are observed in the boundary membrane of sea urchin embryos (Lundgren, 1973). Material is supposedly synthesized for plasma membrane formation in *Amoeba* (Szubinska, 1971) and stored in the cytoplasm as an emulsion of spherical droplets similar in diamter (0.04 to 0.08 μ) and density to those in *P. polymerus* (0.03 μ). These are apparently used by adhering to the inside of the membrane and releasing the products outside to form new membrane. It is possible that the small vesicles in *P. polymerus* eggs are also secreted to form new membrane since they are most active at the constriction rings which may stretch the oolemma (Fig. 55).

Although glycogen reserves are only about 2% (by dry weight) of the more yolky oocytes of an Australian barnacle, *Ibla quadrivalvis* (Woods, 1969), vast aggregations of glycogen granules are found in *P. polymerus* fertilized eggs. According to Barnes (1965), the glycogen reserves in the less yolky *Balanus balanus* and *B. balanoides* embryos, as in *P. polymerus*, are rapidly consumed during development. This is

Figure 56. *Pollicipes polymerus.* The author's conception of a model of the peristaltic constriction event: 1) mitochondria form small multimembranous bodies close to the constriction rings, and smooth endoplasmic vesicles abound, either possibly releasing bound Ca++ to stimulate contraction; 2) microfilaments polymerize from precursors vegetal (V) to an approaching constriction ring, form a network, and orient equatorially just prior to and during constriction in the vegetal part of the egg. After forming a vegetal constriction, the filaments disperse into networks again and depolymerize. Signals from the animal pole (A) pacemaker region initiate peristalsis, after which is a refractory period when signals are either not received or are not of sufficient strength; 3) PC yolk (1) is breaking down within MVBs. MVBs form from aggregated smooth endoplasmic vesicles (2 and 3) which eventually surround and move into the yolk (4 to 6). Internal vesicles are then dispersed into the cytoplasm (5 and 6), some possibly constituting dense bodies and others becoming the border spheres. Glycogen and PC yolk are likely energy sources for the peristaltic movement.


A
V
V

consistent with the observation that glycogen granules may enter MVBs in *P. polymerus* eggs (Fig. 44F).

Some mitochondria were still intact after treatment with 550 μg/cm^3 antimycin A for 90 min, although most had reduced cristae and accumulations of electron dense inclusions (Fig. 53A) as in some metabolically-stressed cells (Abolins-Krogis, 1970). Thus, it is possible that some oxidative phosphorylation may be used for peristaltic constriction, although it is likely that mitochondrial function is impaired after a short period. Budding of mitochondria is also commonly observed in *P. polymerus* constricting eggs which may be important in their duplication (Norrevang, 1968) and suggests activity.

Since PC yolk and glycogen granules may be digested in MVBs adjacent to constriction rings, they are likely the energy sources, and since antimycin A treatment disrupted most mitochondria while peristalsis continued, a large proportion of the energy may be generated by anaerobic glycolysis. Kamiya (1960) found that glycolysis is easily used for generation of cytoplasmic streaming in plant cells, while respiration gains significance as an energy source only when released readily from the mitochondria and is able to contact the mechanochemical system of contractile protein. Eastman (1968) suspected that the predominant pathway of glucose degradation in early (1 to 4 cells) *P. polymerus* embryos was the pentose shunt.

According to the results of Lewis *et al.* (1973), some protein synthesis is probably necessary for peristaltic constriction. In some other contractile cells, protein synthesis is not necessary for microfilament function (Goldman *et al.*, 1973). However, microfilaments are probably constantly polymerizing and depolymerizing, and aggregating into networks and arrays and then disaggregating in *Pollicipes* eggs. Thus, due to the nature of peristalsis, the rates of synthesis and turnover of contractile proteins may be high. The pools of precursors may not be large enough to continue for hours of peristalsis.

Possible Mechanisms for Propagating Peristaltic Constriction Rings

It is obvious that the peristaltic system under investigation requires 3 phases: 1) stimulation from a pacemaker zone near the animal pole, 2) contraction of cortical microfilaments and 3) relaxation possibly due to a refractory period when stimulation is inhibited; much like the generation of a membrane potential.

Although cytokinesis and polar lobe formation are probably not controlled by the nucleus since the cleavage rate in sea urchins is determined exclusively by the cytoplasm (Moore, 1933) and because polar lobe formation occurs in enucleated eggs in synchrony with nucleated eggs (Verdonk *et al.*, 1971), nuclear material or the associated mitotic apparatus during polar body formation may be associated with peristaltic constrictions since the female pronucleus resides at the animal pole during peristaltic constriction in *Pollicipes* (Nussbaum, 1890).

Schroeder (1969) and Tilney and Marsland (1969) suggested that nuclear breakdown and/or annulate lamellae formation was necessary for microfilament formation and function. Moreover, HMM-binding filaments have been detected in the mitotic apparatus of mammalian cells, suggesting a possible cytoplasmic pool of filaments or precursors prior to cleavage (Hinkley and Telser, 1974).

Another possible method of control of peristalsis may be the accumulation of the oviducal gland's activating fluid which stimulates oocyte nuclei to pass into metaphase (Walley *et al.*, 1971).

According to Sawai and Yoneda (1974), the waves of stiffness in the newt egg, which begin at the animal pole, may be controlled by a stimulating substance released from the mitotic apparatus before cleavage and spread along the cortex. Timourian *et al.* (1974) implicated Ca^{++} as the stimulating or determining substance released from mitotic centers.

In several contractile systems, thin microfilaments are closely allied with small vesicles which may hold local Ca^{++} pools (Woollacott, 1974). In the case of control of *Ilyanassa*'s polar lobes, Conrad and Williams (1974) suggest the ratio of intracellular Ca^{++} to K^{+} is critical. In fact, according to the postulates of this theory, intracellular Ca^{++} is sequestered until released periodically in response to specific stimuli. It is further suggested that possible sites of Ca^{++} accumulation include mitochondria (Lehninger, 1964) and multimembranous vesicles (Crowell, 1964; Bluemink, 1970; Gerin, 1972), such as those regularly formed in contracting *Pollicipes* eggs (Figs. 43B and C) and observed in *Dentalium* polar lobes (Reverberi, 1970). Schroeder and Strickland (1974) found that ionophores, which release stored Ca^{++} from ER vesicles and mitochondria, stimulated cortical contractions in frog eggs with or without extracellular Ca^{++} and further, that intracellular EDTA or EGTA inhibits contractility, suggesting that intracellular Ca^{++} is important in contraction (see also Conrad, 1974).

An immediate consequence of the cortical reaction at fertilization in *Xenopus* eggs may be an increase of intracellular Ca^{++} concentration (Wolf, 1974), which may activate specific enzyme systems as well as cortical contractile systems (Epel *et al.*, 1969; Morrill *et al.*, 1971), When Ca^{++} was injected immediately under the plasma membrane of amphibian eggs (Gingell, 1970), local contractions of a microfilament network below the membrane (Wessells *et al.*, 1971) ensued. Thus, it is felt that Ca^{++} is important in its control. Gingell (1970) further speculated that a change in plasma membrane permeability triggered by adsorption of charged molecules alters surface potential and allows Ca^{++} influx. This may be physiologically significant since higher intracellular Ca^{++} concentrations have been measured at the cleavage furrow during metaphase than at the poles (Timourian *et al.*, 1974).

An alternative hypothesis (Rosenberg, 1967) as related to furrow formation (Bluemink, 1970) is that a change in chemical potential may lead to mechanical deformation which in turn changes chemical potential, and is thus self-propagating. In the case of gut peristalsis, the mechanical distention is responsible for decreasing excitability, thus continuous nervous stimulation is necessary (Davson, 1964). Hoffman-Berling (1958) first suggested that the motive force for ciliate contraction is associated with a configurational change in the contractile filaments brought about by the direct charge interaction of Ca^{++} with reactive sites along their length.

Possible Function of Peristaltic Constriction Rings

Three possible functions were discussed earlier: 1) ooplasmic segregation of lipid yolk, 2) lifting of the egg membrane and 3) egg elongation (Lewis *et al.*, 1973). Although Groom (1892) noted constriction rings ceased only after the yolk and cytoplasm separated in *Lepas* eggs, they were not suggested as the motive force of ooplasmic segregation by Lewis *et al.* (1973) since 1) no segregation was observed at Stages I and II when the rates of constriction were highest and 2) when the constriction is stopped by CCB, ooplasmic segregation continues. However, there may be a step-wise control of segregation as in *Ilyanassa* polar lobe formation such that only the fastest moving peristaltic constriction rings (Stages I and II) are important in controlling lipid segregation. As early stages were not tested with CCB, this possibility can not be discarded. It is well known that endoplasmic thin filaments (Nagai and R[redacted] 1966; Allen, 1974) or microtubules (Rebhun, 1967; Porter, 1973) may generate the forces necessary in various plant and animal cell streaming and saltatory particle movement. However, these filaments normally run in the direction of streaming (Allen, 1974).

Lifting of the egg membrane may be related to contractile action, as the membrane first becomes visible along the constriction ring in Stage III embryos (Fig. 32). But there is no ultrastructural evidence to further this theory. The only differences noted between oolemmas in a constriction ring and those not in a ring are that the oolemma folds up, producing irregular microvilli and more extracellular spheres are observed in a constriction ring. In fact, the egg membrane lies against the oolemma again after a constriction ring passes by.

It is likely that egg elongation is accomplished by the peristalsis as the increase of the peristaltic velocity at the middle of the egg during Stage III corresponds to the time of greatest observed elongation; the gradual slowing of the constriction movement corresponds to the completion of elongation; and when the peristaltic constrictions are stopped by CCB at Stage I or II, the elongation also stops. In contrast, other cellular elongation is linked to microtubules (Schroeder, 1971), but since microtubules are not found in large numbers during the time of observed egg elongation, and colchicine does not block peristalsis, microtubules are probably not involved in egg elongation. The observation that when the egg membrane is torn during peristalsis it is followed by egg elongation and concurrent deepening of the vegetal constriction ring more than usual tends to support this contention. Evidence from Byers and Porter (1964) indicates that cell elongation is usually accompanied by a peristaltic motion.

Although experimental results did not elucidate the relationship between the polar body formation and the peristaltic constrictions, it should be stressed that the site of polar body formation (animal pole) is also the area where the constriction rings are generated. This area may be regarded as a "pace-maker" in relation to the peristaltic constrictions.

Another possible function is that the first cleavage furrow may be predetermined by the constrictions. However, constriction rings probably have no relationship with the cleavage furrow, since peristalsis ceases long before first cleavage, and the animal pole "pace-maker" region becomes one side of a bilaterally cleaved furrow; that is, the cleavage furrow forms slightly obliquely to both poles while the egg rotates inside the egg membrane (Bigelow, 1898).

GENERAL DISCUSSION AND CONCLUSIONS

As specific questions are considered in the discussion sections of individual chapters, this discussion is an attempt to tie together the various chapters of this thesis, to compare the results found in this study to those in the literature, and to present summaries and draw some conclusions.

The information is organized as follows: 1) environmental effects on feeding, growth and reproduction of *Pollicipes polymerus*, including differences in reproduction at various latitudes; and 2) descriptive morphology of developmental events.

1) *Environmental Effects*

When the growth rates of control embryos were compared to embryos grown under variously modified circumstances (all maintained in the laboratory), those growth rates were faster where conditions closely resembled those in the adult barnacle mantle cavity. However, addition of antimicrobial drugs and division of the egg lamellae into small pieces also promoted growth and development.

Nauplii were fed many species of algae; only 3 species promoted growth to Stage 5 or older. These algae affected growth more in combination than when alone. Naupliar growth was fastest in larvae fed the combination of *Prorocentrum micans/Platymonas* sp. On the other hand, larvae fed a *Prorocentrum micans*/pennate diatom diet grew slowly, and most larvae died at naupliar Stage 3. Interactions between algal species may have affected their nutritional value for the nauplii (Fig. 21).

Only the growth of larvae eating *Prorocentrum micans* depended upon the larva-to-food ratio. That is, animals given more food per larva grew faster from stage to stage than those with less food. Thus, the concentration of algae chosen for the other diets was probably close to optimum. A feeding schedule is proposed which should theoretically promote optimal growth based upon data collected in this study: 500 cells/mm^3 of *Platymonas* sp. alone up to 9 to 10 days after hatching; 5 to 10 cells/mm^3 of *Prorocentrum micans* is then added to the above until naupliar Stage 6 is reached, at which time the *Platymonas* sp. concentration is drastically reduced or even eliminated and nauplii are maintained on *Prorocentrum* alone.

Although growth rates varied little between the adults in the field, an ascending order of mean adult size occurs from animals at Edward's Reef high intertidal level, Edward's Reef low, Eagle Point high to Eagle Point low (Fig. 57). Relative juvenile growth rates in the 4 populations follow a similar trend, although the data for Edward's Reef low animals overlap those for Eagle Point high animals (Fig. 58). This trend given above is also observed when percent brooding in average months (Fig. 59) and mean monthly egg production (Fig. 60) is compared between populations. It is noteworthy that in Figures 57 to 60 the lines drawn through the 2 points at Eagle Point and the 2 points at Edward's Reef have nearly the same slope. When placed in juxtaposition, the abscissa depicting the Edward's Reef populations is displaced by 5 feet of elevation in relation to the Eagle Point populations (Fig. 57). Thus, one might say that some factor(s) at Eagle Point have the equivalent effect on adult size as a decrease in intertidal height of 5 feet at Edward's Reef. These physical or biological factors may affect juvenile growth rate at Eagle Point in the same manner as a decrease in elevation

Figure 57. *Pollicipes polymerus.* Average adult size at 2 intertidal heights at Eagle Point and Edward's Reef. Standard deviations are indicated.

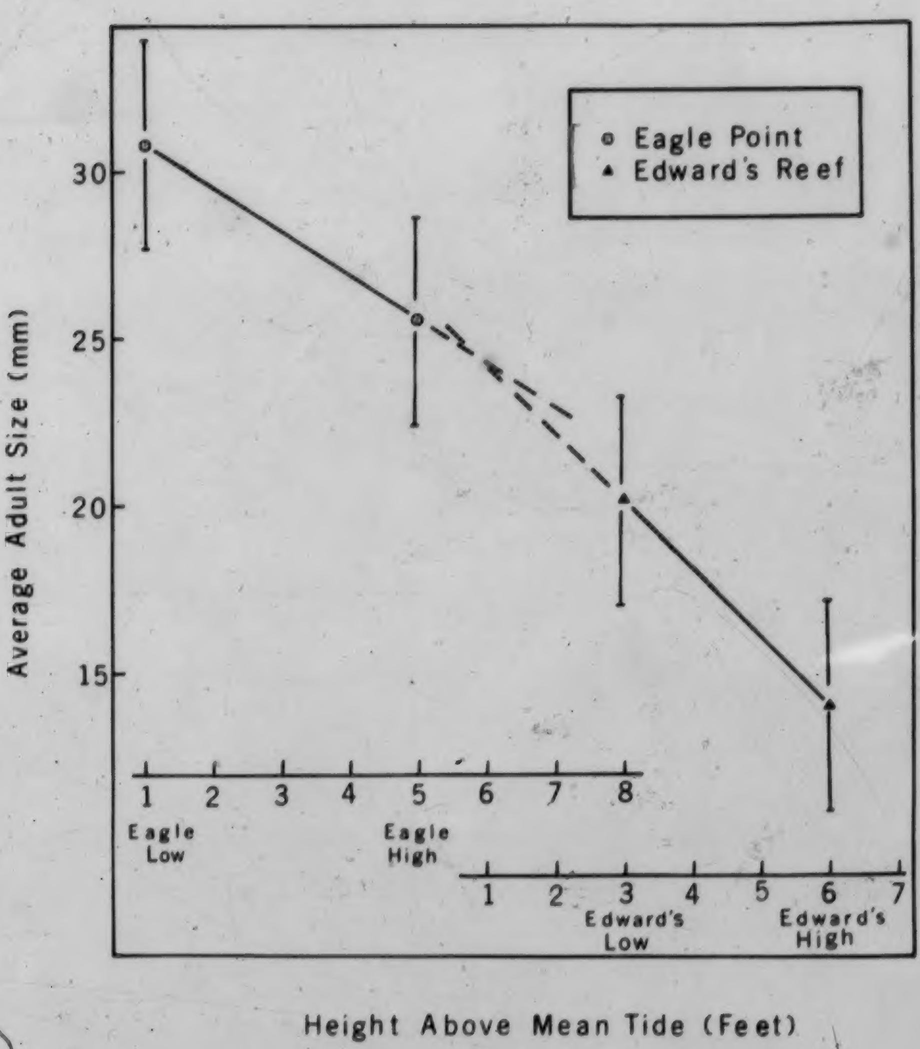

○ Eagle Point
▲ Edward's Reef
Average Adult Size (mm)
30
25
20
15
1
2
3
4
5
6
7
8
Eagle Low
Eagle High
1
2
3
4
5
6
7
Edward's Low
Edward's High
Height Above Mean Tide (Feet)

Figure 58. *Pollicipes polymerus.* Juvenile growth rate at 2 intertidal heights at Eagle Point and Edward's Reef. Standard deviations are indicated.

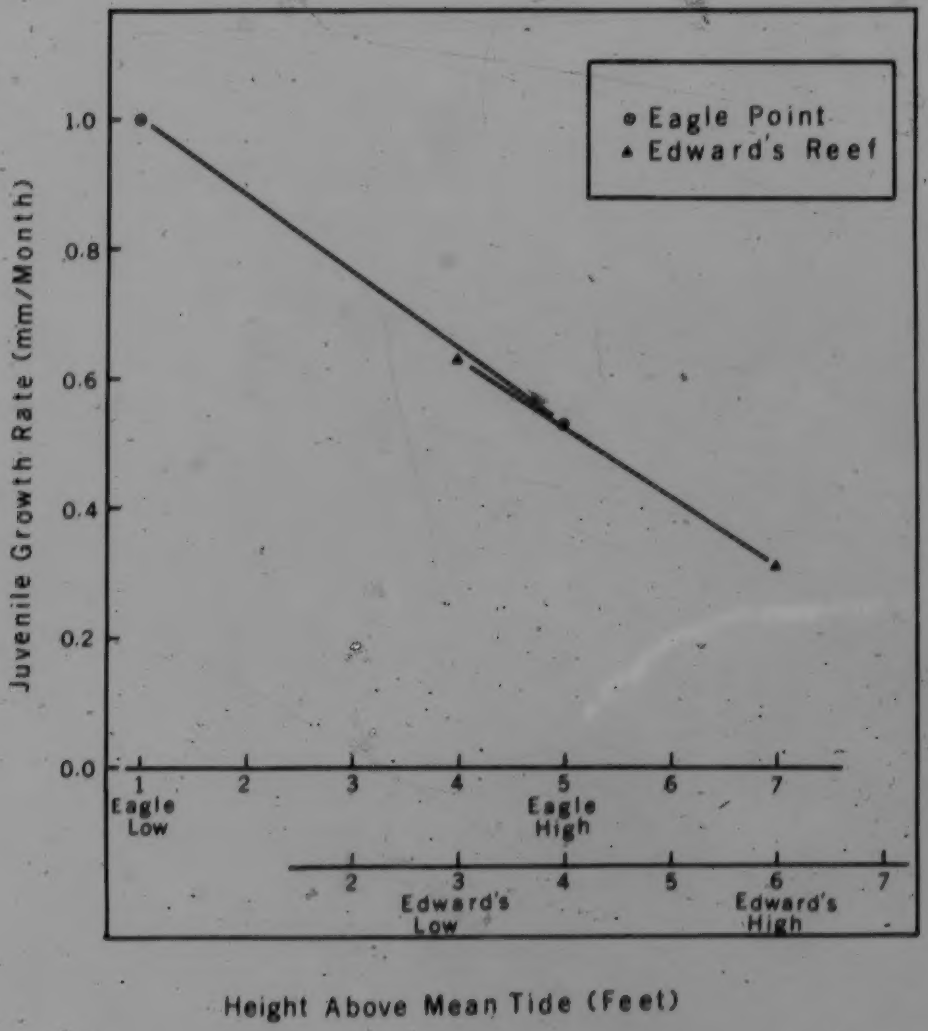

Eagle Point
Edward's Reef
Juvenile Growth Rate (mm/Month)
1.0
0.8
0.6
0.4
0.2
0.0
1
Eagle
Low
2
3
4
5
Eagle
High
6
7
2
3
Edward's
Low
4
5
6
Edward's
High
7
Height Above Mean Tide (Feet)

Figure 59. *Pollicipes polymerus.* Percent brooding in an average month at 2 intertidal heights at Eagle Point and Edward's Reef. Standard deviations are indicated.

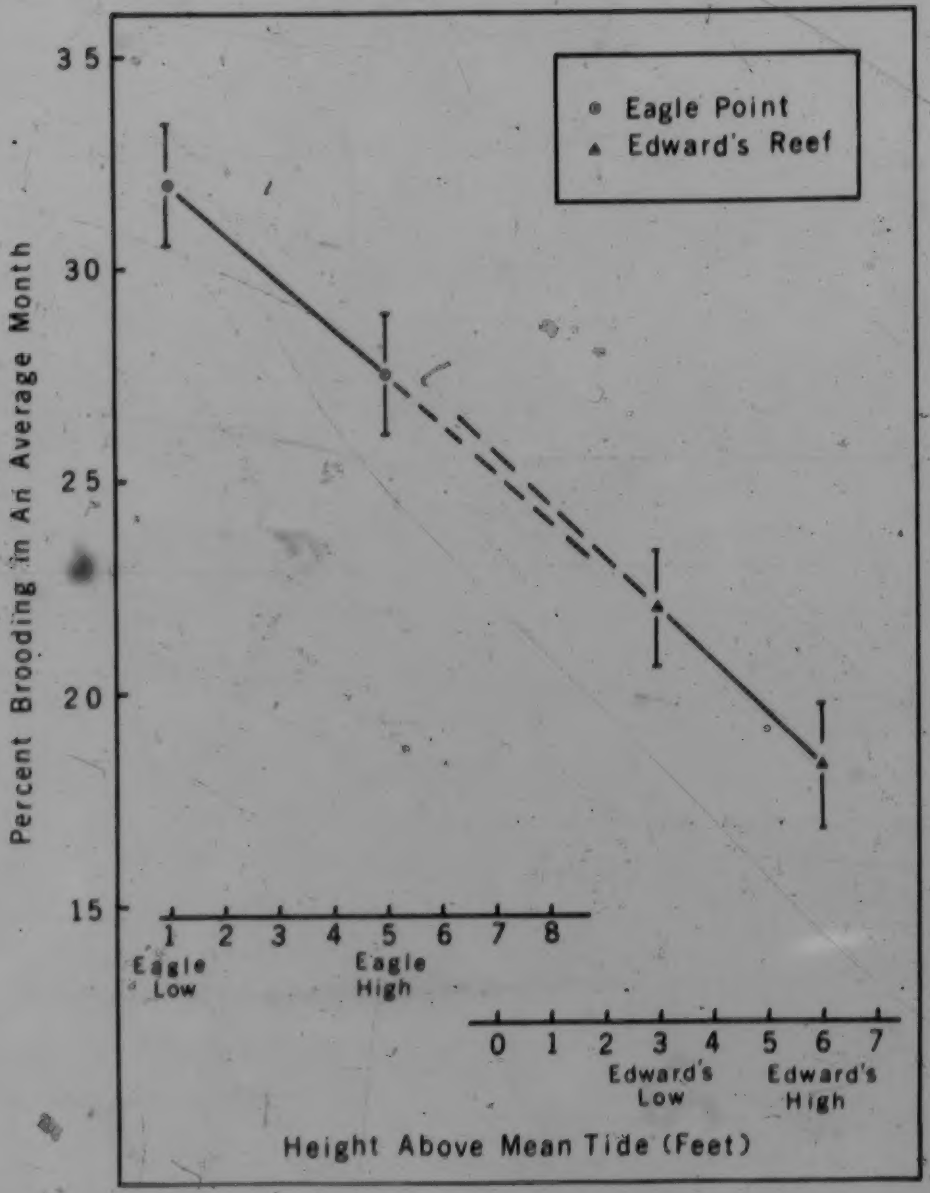
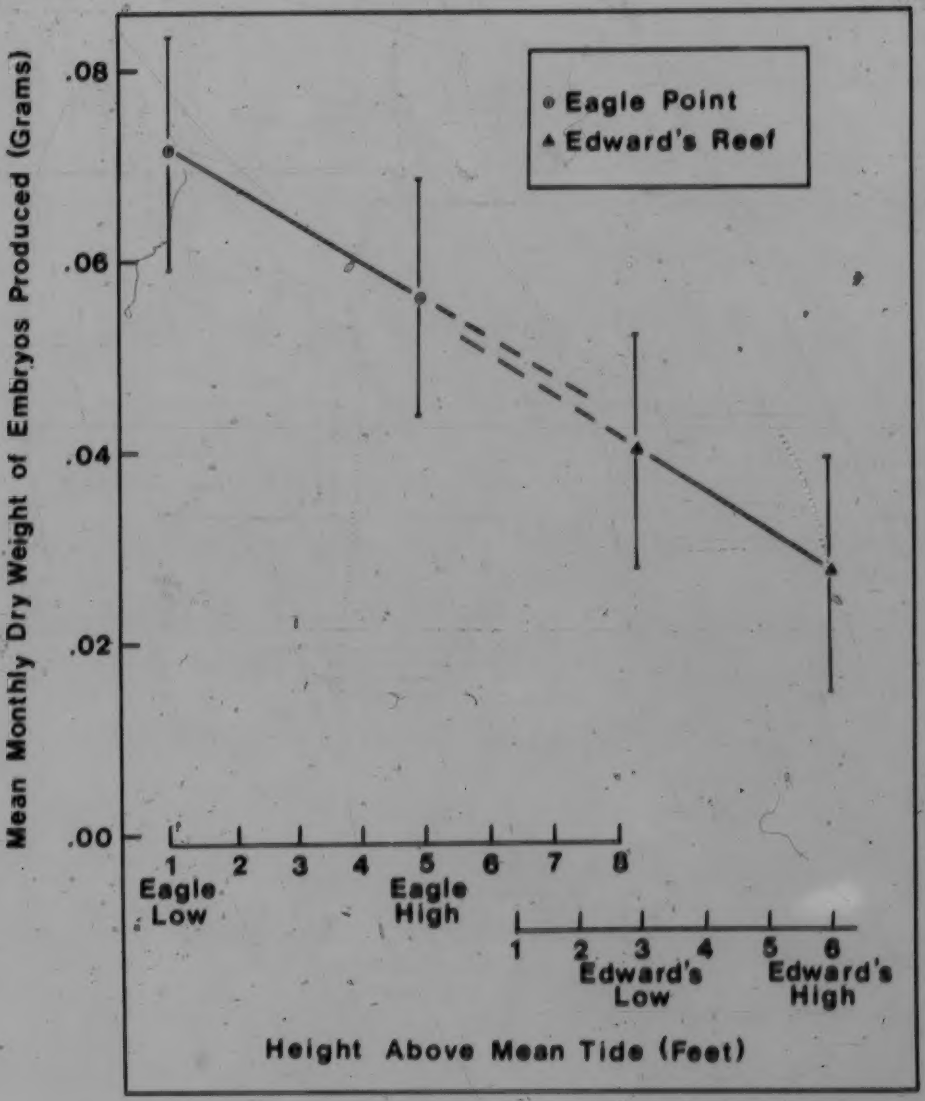

Eagle Point
Edward's Reef
Percent Brooding in An Average Month
35
30
25
20
15
1
2
3
4
5
6
7
8
Eagle Low
Eagle High
0
1
2
3
4
5
6
7
Edward's Low
Edward's High
Height Above Mean Tide (Feet)

Figure 60. *Pollicipes polymerus.* Mean monthly egg production (times 10) at 2 intertidal heights at Eagle Point and Edward's Reef.


Mean Monthly Dry Weight of Embryos Produced (Grams)
.08
.06
.04
.02
.00
● Eagle Point
▲ Edward's Reef
1
2
3
4
5
6
7
8
Eagle Low
Eagle High
1
2
3
4
5
6
Edward's Low
Edward's High
Height Above Mean Tide (Feet)

of 1 foot at Edward's Reef (Fig. 58). Likewise, a decrease of 7 feet at Edward's Reef is necessary to match the effect on reproductive activity (Fig. 59), and a decrease of about 5 feet for average monthly embryo mass weight (Fig. 60). The effect observed on adult size is greater than on juvenile growth rate, and may be cumulative. The most pronounced effects, however, are on reproductive activity (Fig. 59) and on fecundity (Fig. 60).

Some obvious environmental factors to be considered which may affect reproduction and fecundity are wave action, habitat, fluctuation of salinity, and food supply. *P. polymerus* broods for a longer period and more intensively at Eagle Point than at Edward's Reef. In addition, more *P. polymerus* were brooding in June, 1972, at Windmill Beach (north of San Francisco) than at Doran Beach, not more than 5 miles away. Optimum conditions for reproduction appear to be heavy surf, rocky habitat, constant salinity, and abundant food. Less than optimal rates of brooding occur when barnacles are anchored in the "cave-like" habitat with fresh water run-off at Edward's Reef or on the boulders which emerge occasionally from the sandy Doran Beach. Sand scours the barnacles' plates at Doran Beach and may be found within their mantle cavities. *Zostera marina* also fouls the barnacles at Doran Beach.

Adult barnacles exposed to greater wave action (Eagle Point) are apparently stimulated to feed more often due to the splash and force of the waves and thus, they probably obtain more food than those at Edward's Reef. Because *P. polymerus* has no cirral beat as an adult, it relies primarily upon currents to bring in food (Barnes and Reese, 1959; Howard and Scott, 1959).

Due to the large changes in tidal level at San Juan Island, the currents near shore tend to be very strong, reaching 9.3 Km/h at Cattle Point on the southern tip of San Juan Island (Connell, 1970). Thus, large local effects on wave action are likely, such as the refraction of waves by currents, with a consequent change in the wave-induced currents (Fairbridge, 1966, p. 588-590). Shelford and Towler (1925) also noted that wave action during the summer is important to the upper intertidal inhabitants on the west side of San Juan Island, allowing frequent immersion.

The force and duration of wave action may influence *P. polymerus* populations by: 1) effects upon respiration, which is undoubtedly affected by the oxygenation of the water, 2) abundance of food, which is certainly related to fast currents and upwelling, and 3) stimulation of extension feeding activity in adults, which in turn depends upon the force of the waves or currents. Since much respiratory gas exchange apparently occurs directly from air through the peduncle (Petersen *et al.*, 1974), food availability and feeding stimulation probably play a greater role in differential growth and reproduction.

Batham (1945) records finding *P. polymerus* in "violently surging waves," mostly at the half tide level, although it may extend to low and high tidal levels in "channels" in New Zealand. It is likely, then, that the genus *Pollicipes* is confined to rocky areas because these areas are exposed to heavy wave action. Apparently, several features of adaptive value have allowed the genus to maintain its position in this habitat: 1) gregarious settlement of cyprids, providing dense populations which facilitate cross-fertilization and mutual protection from the environment, 2) cirral beating behavior of juveniles newly-settled on adult stalks, which utilize a microfeeding method when competing with non-cirral-beating macrophagous adults, and 3) multi-directional orientation of capitula in large clusters of adults which serves to maximize the feeding efficiency of each individual in the group.

The loss of cirral beating with adulthood may dictate the habitat in which this genus can survive. Under these circumstances, efficiency would depend upon activity of the sea water (Batham, 1945). The question: "Which came first, the behavior which is adapted to the habitat, or the inclination to settle in a favorable habitat?" cannot be answered from the information at hand. Cirral extension feeding behavior may have been selected for by the habitat, the animals using cirral extension in calm waters being eliminated.

One other obvious difference between the study sites on San Juan Island concerns variation in the amount of fresh water run-off. Average monthly ocean surface salinity varies only from 28.5 to 29.1% (Connell, 1970); however, average monthly rainfall varies from 1.0 to 14.1 inches (Johnson and Thompson, 1927-31). The high tidal pool above the *P. polymerus* population studied at Edward's Reef collects fresh water run-off during most of the year, including part of the summer (dry season is from May to mid-August). The shortened reproductive period at this site may be partly due to physiological stress since *P. polymerus* regulates hydrostatic pressure of the haemolymph and intracellular volume (Fyhn *et al.*, 1972).

Barnes and Reese (1960) felt that the San Juan Islands present an exception to the animal's common habitat, since they are supposedly more protected than the outer coast. Rice (1930), Towler (1930), Henry (1940a,b), and Barnes and Reese (1960) observed the species above low tide, extending to higher levels in crevices in the San Juan Islands than is usually found on the outer coast.

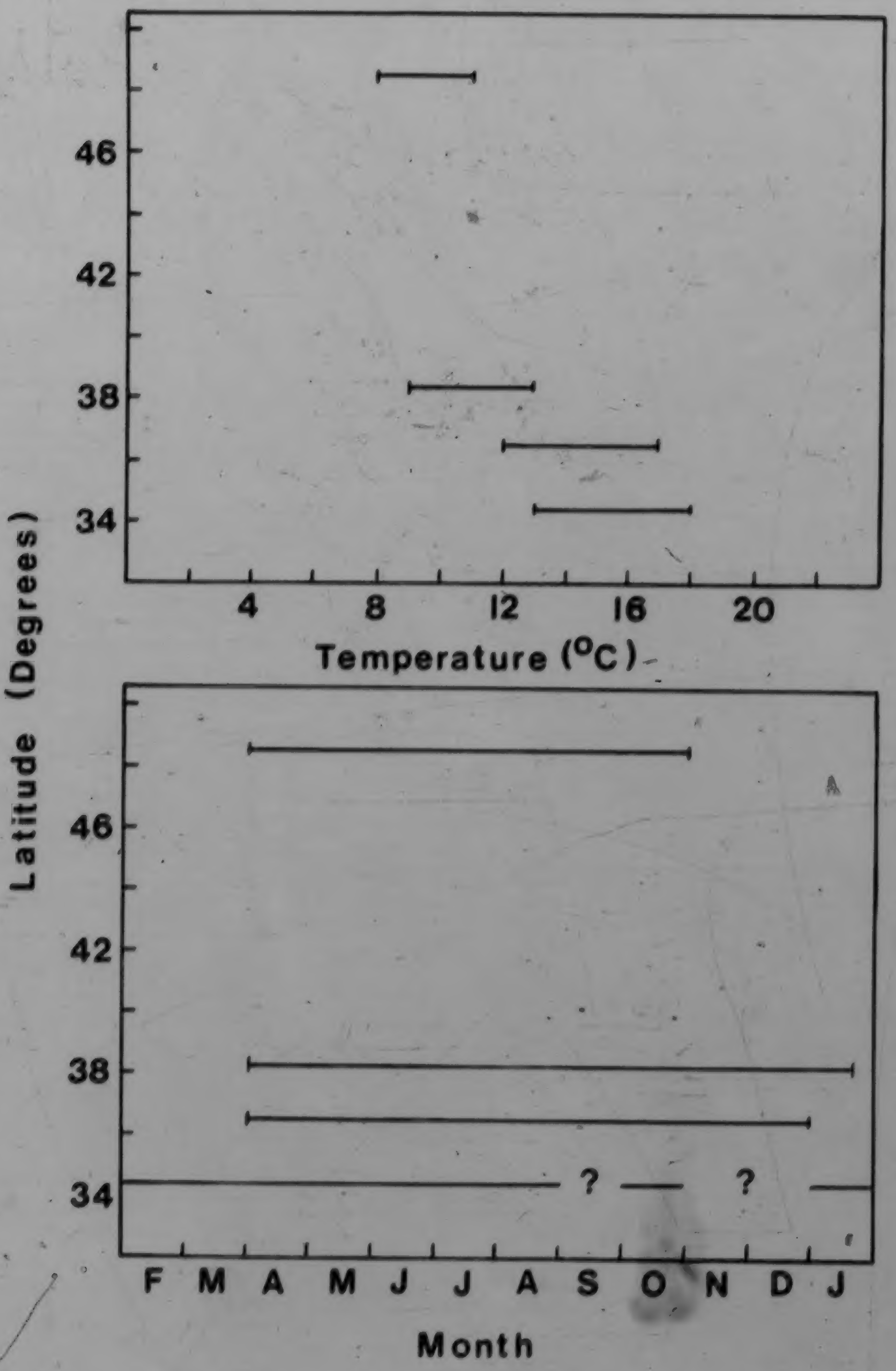
Some barnacle species exhibit a progressive delay of the onset of the breeding season from southern to northern habitats (Hutchins, 1947). This phenomenon supports the theory that water temperature is an important factor affecting the onset of breeding in these species. In contrast, the brooding period in *P. polymerus* extends for more of the year as one goes south (Fig. 61). However, the brooding cycle at any one latitude follows the fluctuations of the sea water temperature (e.g., Hilgard, 1960), and measurements show an overlapping of temperatures during the reproductive period from north to south (Fig. 61).

According to Straughan (1971), *P. polymerus* at Santa Barbara brood throughout the year; however, the data given are not sufficient to support this claim.

First, Straughan justifies the use of only 10 animals sampled on a monthly basis by saying "At each station, there was only a small variation in the fraction brooding eggs in samples of different sizes." Yet, in April, 1969, she reports a range of 10 to 90% lightly-oiled (defined as "a thin film" of oil) *P. polymerus* brooding at Goleta Point (presumably a single station) and speculates that the great variation is due to oil effects. Had the range of variation among clean (oil-free) animals been given, or a larger sample size employed (e.g., 20 to 30), this view might be more credible.

Although no brooding data are given for November or December, and only for moderately-oiled animals in September, she suggests that *P. polymerus* reproduces all year at Santa Barbara.

Figure 61. *Pollicipes polymerus.* Variation in mean surface sea water temperature and reproductive period with latitude.


46
42
38
34
4
8
12
16
20
Temperature (°C)
Latitude (Degrees)
46
42
38
34
?
?
F
M
A
M
J
J
A
S
O
N
D
J
Month

Lastly, Straughan reported only 100 embryos produced per adult, while 100,000 to 200,000 are reported by Hilgard (1960) and in the present study. It would be most interesting to discover if the individual embryos are the same size at each locality, or if the southern ones are larger. Further investigations, including fecundity data, are needed to clarify these apparently conflicting reports.

2) *Developmental Events*

During the maturation of fertilized eggs, an unusual event was observed: a series of constriction rings move slowly from the animal to the vegetal pole. Since various contractile phenomena are now considered to be controlled by microfilaments, the peristaltic constrictions present a new system in which to explore this hypothesis.

When originally observed, the peristaltic constriction phenomenon in barnacle eggs was suggested by Bigelow (1902) to represent a "modified" polar lobe. A brief comparison of peristaltic constriction and polar lobe formation is pertinent here, since at the light microscope level, the changes in cell shape are quite similar, though clearly distinct. According to the categories listed by Conrad (1973, Tables 2 and 3), peristaltic constrictions are similar to polar lobes in *Ilyanassa* (prosobranch gastropod) in the following ways: 1) general morphology, 2) association with an equatorial ring of microfilaments (seen at least in vegetal peristaltic constrictions), 3) cytochalasin B-sensitivity, 4) no observed association with cleavage asters, 5) incomplete partitioning of the cytoplasm, 6) relaxation after a period of maximal constriction, and 7) maintenance over substantial periods of time of contraction (maintenance of sequential peristaltic rings, not single rings, in the case of *Pollicipes*). In addition to these similarities, glycogen granules, mitochondria and PC yolk are closely adjacent to polar lobe constrictions (see micrographs, Conrad and Williams, 1974a,b; Conrad *et al.*, 1973). The presence of MVBs was not discussed, although Pucci-Minafra *et al.* (1969) observed them in the polar lobes.

Peristaltic constrictions are not inhibited by colchicine (Lewis *et al.*, 1973), while colchicine-sensitive steps are involved in polar lobe formation. Several rates of constriction movement are observed, according to the constriction stage (Lewis *et al.*, 1973), rather than 2 rates only as in *Ilyanassa* polar lobe formation. No microfilament network is reported in *Ilyanassa* polar lobes, although circumferential microfilaments may have obscured a network if it were present.

Polar lobes and peristaltic constrictions are precisely-timed events. The presumed function of polar lobe formation is to assure that all or most of the morphogenetic determinates lodged in the polar lobe cortex are passed on to a particular blastomere during a cleavage. However, this does not explain why, in *Ilyanassa*, polar lobes also form at both meiotic divisions. Peristaltic constriction, on the other hand, occurs only between the meiotic divisions in *P. polymerus*.

Since Schroeder's (1968) discovery that circumferentially arranged thin microfilaments in contractile rings are probably the morphological basis of cytokinesis in the *Arbacia* egg, many such studies have confirmed Schroeder's finding in other cells. The primary difference between contractile rings and peristaltic constriction rings is that the latter are not stationary, but move along the egg's long axis. The wave of peristaltic movement occurs at a rate of 14 μ/min in *P. polymerus*. This rate is well within the range of rate of contraction inward measured during cytokinesis (6 μ/min: Tucker, 1971 to 40 μ/min: Schroeder, 1973). The *Arbacia* egg, whose diameter (75 μ) corresponds most closely (of those eggs measured) to that of the *P. polymerus* egg (85 μ) has a contractile ring width of 3 to 17 μ; constriction ring width in *P. polymerus* averages 6 μ. However, the thickness of all contractile rings measured is consistently 0.1 to 0.2 μ, while that of the *P. polymerus* constriction ring measured up to 0.5 μ. Average microvillar thickness in vegetal constriction rings is 0.9 μ, which added to 0.5 μ, totals 1.4 μ. Perhaps this greater thickness is related to the movement of the constriction in 2 planes rather than 1.

Several moving contractile phenomena in eggs are compared in Table 22. Little is known of the nature of the *Nereis* egg movement, except that it is slow with shallow contractions and lasts about 100 min. Of the phenomena listed, the surface contraction waves in salamander eggs (Hara, 1971) appear to be most similar to the peristaltic movement described here in *P. polymerus* eggs. The salamander egg waves move about 25 μ/min (based upon a 2.5 mm diameter egg; Bluemink, 1970), which compares favorably with 14 μ/min in *P. polymerus* (still within the range of the speed of the contractile ring movement). Apparently, the surface contraction waves in ova of salamanders and newts is somehow related to a wave of "stiffness" which passes over them (Sawai and Yoneda, 1974), and both phenomena may be related to the formation of the first cleavage furrow, even though the contractions are equatorial and cleavage is meridional. The important point here is that the cleavage furrow closely follows the "wave" temporally. Bluemink (1970) observed microfilaments in the non-furrow cortex of the *Ambystoma* egg, indicating that the capacity for furrow formation may be related to the presence of cortical filaments. In the case of *P. polymerus*, the first cleavage furrow rotates from the meridional

Table 22. *Comparison of morphogenetic movements in various cells.*

Title (Reference in parentheses)	Description	Possible Function(s)	Suggested Mode of Control or Generation
1. Pulsations in Annelid eggs (*Nereis*). (Hoadley, 1934)	1) Localized irregularities and 2) "pulsations" (incomplete description). Accompanied by egg elongation and pole flattening. Begins at GV breakdown, rests in polar areas during polar body formation, stops before and during cleavave, stops completely before second cleavage.	Cleavage plane formation; no information as to relationship with cleavage plane.	Independent of nucleus (?)
2. Cortical movements in Mollusc eggs (*Barnea*). (Pasteels, 1966)	Undulating waves of chorion.	Lifting and folding of chorion.	Independent of plasma membrane; rhythmic elongation of microvilli correlates with physico-chemical changes affecting egg surface before furrow or polar lobe formation.

Table 22 (continued)

Title (Reference in parentheses)	Description	Possible Function(s)	Suggested Mode of Control or Generation
3. Undulations of chorion in Annelid eggs (*Chaetopterus*). (Pasteels, 1949)	Undulating waves of chorion.	Related to egg maturation, segmentation and furrow formation.	?
4. Surface contraction waves in salamander eggs (*Ambystoma*). (Hara, 1971)	Surface contractions originate at future initiation point of first cleavage furrow and move from animal to vegetal pole.	Related to cleavage furrow formation.	Possibly microfilaments (see Bluemink, 1970).
5. Wave of stiffness in newt eggs during cleavage (*Triturus*). (Sawai & Yoneda, 1974)	Prior to 1st and 2nd cleavage stiffness increases and travels equatorially as a belt as cleavage furrow progresses from animal to vegetal pole; coincides with wave of "furrow-forming activity"	Expression of some preparation necessary for cleavage furrow formation.	?

Table 22 (continued)

Title (Reference in parentheses)	Description	Possible Function(s)	Suggested Mode of Control or Generation
6. Peristaltic waves of thickenings from midbody in cleaving HeLa cells. (Byers & Abramson, 1968)	Unidirectional waves which move from center of midbody at telophase to both cells.	Longitudinal translation of cytoplasm to cells and elongation of midbody.	Microtubules in axis of elongation (no filaments in waves).
7. Peristaltic constriction rings in fertilized barnacle eggs (*Pollicipes*). (Lewis *et al.*, 1973; present study)	Constriction rings travel from animal to vegetal pole between 1st and 2nd polar body formation.	Egg elongation, lifting egg membrane, ooplasmic segregation, cleavage furrow formation.	Cortical network and vegetal para-equatorial microfilaments.

to the equatorial plane (Nussbaum, 1890), so that the furrow is finally perpendicular to the plane of the constriction rings. In addition, there is a lag of about 23 h between the constrictions and the first cleavage (Lewis, 1975b).

In addition to these contractile movements of eggs and embryos (Table 22), which may not be truly peristaltic in nature, several other examples may be cited in invertebrates of morphogenetic movements which are peristaltic. The in-rolling movements of the cricket germ band are peristaltic from anterior to posterior at a rate of 26 to 42 μ/min in the first phase, then slow to 1.2 μ/min in the second phase (Vollmar, 1972). Even cells isolated from the posterior end of the band display pulsating movements *in vitro*. The morphological basis of this event has not yet been clarified.

Blood circulation of the compound ascidian, *Botryllus schlosseri*, also has a peristaltic component. Thin microfilaments have been implicated in the generation of the peristaltic ampullar contractions which force blood into the circumferential tunic vessels, thus functioning as accessory hearts (De Santo and Dudley, 1969). The major differences between this peristalsis and that in the barnacle eggs are that 1) the ampullar wall is multicellular (although nonmuscular) and 2) the ampullar tissue remains contracted until the wave has traveled the length of the ampulla, after which the entire ampulla relaxes. Filaments are primarily arrayed in a circular pattern in the basal parts of the ampullar wall which is a simple epithelium. The filamentous zone ranges from 0.4 to 0.9 μ in depth within the cells, similar to that in vegetal constriction rings (0.5 μ) of *P. polymerus* eggs. The ampullae may reduce their diameter by 25 to 44% in 50 sec, while *P. polymerus* eggs reduce their animal poles by 5% and vegetal poles by 30% or more in a few seconds (Lewis *et al.*, 1973). Filament webs and filaments longitudinal to the cell are found in the ampullar apex. As ampullar elongation does not occur, such filaments may function as antagonists to circular filaments. In contrast to the situation in *P. polymerus* eggs, filaments are found in all parts of the ampullae during contraction; this may be related to the finding that ampullae remain contracted during a single wave rather than relaxing.

A preliminary description of morphological changes during embryonic development of *P. polymerus* was given by Nussbaum (1890): he depicted the embryo during polar body formation, the first cleavage and gastrulation, and the first naupliar stage. Otherwise, information on embryonic and larval development in this species has been lacking, and the present study documents these events. My discovery of the feathery and hispid-type setae on naupliar antennae is interesting as such structures have only been identifed previously in nauplii of *Chthamalus aestuarii*. As larval habitats differ tremendously, this might indicate a common ancestral link between the 2 families Lepadidae and Chthamalidae, now placed in 2 separate suborders, rather than convergence arising from exposure to similar conditions.

P. polymerus cyprids are similar to other barnacle cyprids in size and shape. The only exception is that large frontal oil droplets, thought to serve as a reserve food supply in other species, are not prominent in *P. polymerus*. Thus, it is possible that they either are very short-lived in the plankton or feed. Gut contents of cyprids were not studied. Cyprids may live for 2 to 3 weeks in the laboratory, but this could be due to the lack of the preferred settlement substrate or currents.

Metamorphosis of the larvae of many benthic marine invertebrates is known to be induced by specific external stimuli, such as the presence of food for the adult, the proper substratum, or other members of the same species. With the technique for culturing *Pollicipes polymerus* larvae to the cypris stage now available, settlement in a species of barnacle which is apparently quite specific in its preferences is open to investigation.

LITERATURE CITED

LITERATURE CITED

ABOLINS-KROGIS, A., 1970. Alterations in the fine structure of cytoplasmic organelles in the hepatopancreatic cells of shell-regenerating snail, *Helix pomatis* L. *Z. Zellforsch.* 107: 501-515.

ABRIC, P., 1904. Les premiers stades du developement de la Sacculine (*Sacculina carcini*, Rathke). *Compt. Rend. Acad. Sci.* 139: 430-432.

AIKEN, D. E., 1969. Ovarian maturation and egg laying in the crayfish *Orconectes virilis*: influence of temperature and photoperiod. *Can. J. Zool.* 47: 931-935.

ALLEN, N. S., 1974. Endoplasmic filaments generate the motive force for rotational streaming in *Nitella*. *J. Cell Biol.* 63: 270-287.

ALLISON, A. C., 1973. The role of microfilaments and microtubules in cell movement, endocytosis and exocytosis. Pages 109-148 in: *Ciba Foundation Symposium 14. Locomotion of Tissue Cells*. New York: Elsevier.

ANDERSON, D. T., 1965. Embryonic and larval development and segment formation in *Ibla quadrivalvis* Cuv. (Cirripedia). *Aust. J. Zool.* 13: 1-15.

ANDERSON, D. T., 1969. On the embryology of the cirripede crustaceans *Tetraclita rosea* (Krauss), *Tetraclita purpurascens* (Wood), *Chthamalus antennatus* (Darwin), and *Chamaesipho columna* (Spengler) and some considerations of crustacean phylogenetic relationships. *Phil. Trans. Roy. Soc.* 256: 183-235.

ANDERSON, D. T., 1973. *Embryology and Phylogeny in Annelids and Arthropods*. New York: Pergamon Press. 495 pp.

ANDERSON, E., J. H. LOCHHEAD and E. HUEBNER, 1970. The origin and structure of the tertiary envelope in thick shelled eggs of the brine shrimp, *Artemia*. *J. Ultrastr. Res.* 32: 497-525.

APPELLOF, A., 1912. Invertebrate bottom fauna of the Norwegian Sea and North Atlantic. Pages 457-560 in: *The Depths of the Ocean* (Sir J. Murray and J. Hjort, eds.). Weinheim: J. Cramer.

ARNOLD, J. M., 1967. Fine structure of the development of the cephalopod lens. *J. Ultrastr. Res.* 17: 527-543.

ARNOLD, J. M., 1968. Formation of the first cleavage furrow in a telolecithal egg (*Loligo pealii*). *Biol. Bull.* 135: 408.

ARNOLD, J. M., 1969. Cleavage furrow formation in a telolecithal egg (*Loligo pealii*). I. Filaments in early furrow formation. *J.Cell Biol.* 41: 894-904.

ARNOLD, J. M., 1971. Cleavage furrow formation in a telolecithal egg (*Loligo pealii*). II. Direct evidence for a contraction of the cleavage furrow base. *J. Exp. Zool.* 176: 73-85.

AXLINE, S. G. and E. P. REAVEN, 1974. Inhibition of phagocytosis and plasma membrane mobility of cultivated macrophage by cytochalasin B-role of subplasmalemmal microfilaments. *J. Cell Biol.* 62: 647-660.

BABBAGE, P. C. and P. E. KING, 1970. Post-fertilization functions of annulate lamellae in the periphery of the egg of *Spirorbis borealis* (Daudin) (Serpulidae=Annelida). *Z. Zellforsch.* 107: 15-22.

BAKER, P. C., 1965. Fine structure and morphogenetic movements in the gastrula of the tree frog, *Hyla regilla*. *J. Cell Biol.* 24: 95-116.

BAKER, P. C. and T. SCHROEDER, 1967. Cytoplasmic filaments and morphogenetic movement in the amphibian neural tube. *Dev. Biol.* 15: 432-450.

BALINSKY, B. I. and R. J. DEVIS, 1963. Origin and differentiation of cytoplasmic structures in the oocytes of *Xenopus laevis*. *Acta Embryol. Morph. exp.* 6: 55-108.

BARNES, H., 1952-3. The effect of light on the growth rate of two barnacles, *Balanus balanoides* (L.) and *Balanus crenatus* Brug. under conditions of total submergence. *Oikos* 4: 104-111.

BARNES, H., 1953. An effect of parasitism on *Balanus balanus* (L.) de Costa. *Nature* 172: 128.

BARNES, H., 1955a. Further observations on rugophilic behavior in *Balanus balanoides* (L.). *Videsnk. Meddr. danske naturh.-Foren. Kbh.* 117: 341-348.

BARNES, H., 1955b. Growth rate of *Balanus balanoides* (L.). *Oikos* 6: 109-113.

BARNES, H., 1955c. The hatching process in some barnacles. *Oikos* 6: 114-123.

BARNES, H. 1956. Surface roughness and the settlement of *Balanus balanoides* (L.). *Arch. Soc. Zool.-bot. Fenn. Vanaimo.* 10: 164-168.

BARNES, H., 1957. Processes of restoration and synchronization in marine ecology: The spring diatom increase and the "spawning" of the common barnacle *Balanus balanoides* (L.). *Année Biol.* 33: 67-85.

BARNES, H., 1958. The growth rate of *Verruca stroemia* (O. Müller). *J. Mar. Biol. Assoc.* 37: 427-433.

BARNES, H., 1959a. Stomach contents and micro-feeding of some common cirripedes. *Can. J. Zool.* 37: 231-236.

BARNES, H., 1959b. Temperature and the life cycle of *Balanus balanoides* (L.). Pages 234-245 in: *Friday Harbor Symposia in Marine Biology. I. Marine Boring and Fouling Organisms* (D. L. Ray, ed.). Washington: University of Washington Press.

BARNES, H., 1962. The composition of the seminal plasma of *Balanus balanus*. *J. Exp. Biol.* 39: 345-351.

BARNES, H., 1963a. Light, temperature and the breeding of *Balanus balanoides*. *J. Mar. Biol. Assoc.* 43: 717-727.

BARNES, H., 1963b. Organic constituents of the seminal plasma of *Balanus balanus*. *J. Exp. Biol.* 40: 587-594.

BARNES, H., 1965. Studies in the biochemistry of cirripede eggs. I. Changes in the general biochemical composition during development of *Balanus balanoides* and *Balanus balanus*. *J. Mar. Biol. Assoc.* 45: 321-339.

BARNES, H. and M. BARNES, 1954. The general biology of *Balanus balanus* (L.) da Costa. *Oikos* 5: 63-76.

BARNES, H. and M. BARNES, 1956a. The formation of the egg mass of *Balanus balanoides* (L.). *Arch. Soc. Zool.-bot. Fenn. Vanaimo* 11: 11-16.

BARNES, H. and M. BARNES, 1956b. The general biology of *Balanus glandula* Darwin. *Pac. Sci.* 10: 415-430.

BARNES, H. and M. BARNES. 1959a. The effect of temperature on the oxygen uptake and rate of development of the egg masses of two common cirripedes, *Balanus balanoides* (L.) and *Pollicipes polymerus* J. B. Sowerby. *Kieler Meeresforsch.* 15: 242-251.

BARNES, H. and M. BARNES, 1959b. The naupliar stages of *Balanus nubulis* Darwin. *Can. J. Zool.* 37: 15-23.

BARNES, H. and M. BARNES, 1959c. Note on stimulation of cirripede nauplii. *Oikos* 10: 19-23.

BARNES, H. and M. BARNES, 1963. *In vitro* development of cirripede eggs. *Vidensk. Meddr. danske naturh.-Foren. Kbh.* 125: 93-100.

BARNES, H. and M. BARNES, 1965. Egg size, nauplius size and their variation with local, geographical and specific factors in some common cirripedes. *J. Anim. Ecol.* 34: 391-402.

BARNES, H. and M. BARNES, 1967. The effect of starvation and feeding on the time of production of egg masses in the boreo-arctic cirripede *Balanus balanoides* (L.). *J. Exp. Mar. Biol. Ecol.* 1: 1-6.

BARNES, H. and M. BARNES, 1968. Egg numbers, metabolic efficiency of egg production and fecundity; local and regional variations in a number of common cirripedes. *J. Exp. Mar. Biol. Ecol.* 2: 135-153.

BARNES, H., M. BARNES and D. M. FINLAYSON, 1963. The seasonal changes in body weight, biochemical composition and oxygen uptake of two common boreo-arctic cirripedes, *Balanus balanoides* (L.) and *Balanus balanus* da Costa. *J. Mar. Biol. Assoc.* 43: 185-211.

BARNES, H. and J. D. COSTLOW, JR., 1961. The larval stages of *Balanus balanus* (L.) da Costa. *J. Mar. Biol. Assoc.* 41: 59-68.

BARNES, H. and D. J. CRISP, 1956. Evidence of self-fertilization in certain species of barnacles. *J. Mar. Biol. Assoc.* 35: 631-639.

BARNES, H. and R. M. C. DAWSON, 1966. A note on the lipids of *Balanus* spermatozoa. *J. Mar. Biol. Assoc.* 46: 263-265.

BARNES, H. and R. EVENS, 1967. Studies in the biochemistry of cirripede eggs. *J. Mar. Biol. Assoc.* 47: 171-180.

BARNES, H. and D. M. FINLAYSON, 1962. Presence of ascorbic acid in cirripede semen. *Limnol. Oceanogr.* 7: 98.

BARNES, H. and J. J. GONOR, 1958. Neurosecretory cells in the cirripede *Pollicipes polymerus* J. B. Sowerby. *J. Mar. Res.* 17: 81-102.

BARNES, H. and H. T. POWELL, 1953. The growth of *Balanus balanoides* (L.) and *Balanus crenatus* Brug. under varying conditions of submersion. *J. Mar. Biol. Assoc.* 32: 107-127.

BARNES, H. and E. S. REESE, 1959. Feeding in the pedunculate cirripede *Pollicipes polymerus* J. B. Sowerby. *Proc. Zool. Soc. London* 132: 569-585.

BARNES, H. and E. S. REESE, 1960. The behavior of the stalked intertidal barnacle *Pollicipes polymerus* J. B. Sowerby, with special reference to its ecology and distribution. *J. Anim. Ecol.* 29: 169-185.

BARNES, H. and R. L. STONE, 1972. Suppression of the penis development in *Balanus balanoides* (L.). *J. Exp. Mar. Biol. Ecol.* 9: 303-309.

BASSINDALE, R., 1936. The developmental stages of three English barnacles *Balanus balanoides* (Linn.), *Chthamalus stellatus* (Poli) and *Verruca stroemia* (O. F. Müller). *Proc. Zool. Soc. London* 1: 57-74.

BATHAM, E. J., 1945. *Pollicipes spinosus* Quoy and Gaimard. I. Notes on biology and anatomy of adult barnacle. *Trans. Roy. Soc. New Zealand* 74: 359-374.

BATHAM, E. J., 1946. *Pollicipes spinosus* Quoy and Gaimard. II. Embryonic and larval development. *Trans. Roy. Soc. New Zealand* 75: 405-418.

BATHAM, E. J. and J. T. TOMLINSON, 1965. On *Cryptophialus melampygos* Berndt, a small boring barnacle of the order Acrothoracica abundant in some New Zealand molluscs. *Trans. Roy. Soc. New Zealand* 7: 141-154.

BEAMS, H. W., 1964. Cellular membranes in oogenesis. Pages 175-219 in: *Cellular Membranes in Development* (M. Locke, ed.). New York: Academic Press Inc.

BEAMS, H. W. and R. KESSEL, 1963. Electron microscope studies on developing crayfish oocytes with special references to the origin of yolk. *J. Cell Biol.* 18: 621-649.

BENNETT, H. S. and J. H. LUFT, 1959. s-Collidine as a basis for buffering fixatives. *J. Cell Biol.* 6: 113-117.

BERNARD, F. J. and C. E. LANE, 1962. Early settlement and metamorphosis of the barnacle *Balanus amphitrite niveus*. *J. Morph.* 110: 19-39.

BIGELOW, M. A., 1898. Notes on the first cleavage of *Lepas*. *Zool. Bull.* 2: 173-177.

BIGELOW, M. A., 1902. The early development of *Lepas*. A study of cell lineage and germ layers. *Bull. Mus. Comp. Zool. Harvard College* 40: 61-144.

BLOM, S.-E., 1965. *Balanus improvisus* Darwin on the west coast of Sweden. *Zoologiska Bidrag Fran Uppsala, Uppsala Univ.* 37: 59-76.

BLUEMINK, J. G., 1970. The first cleavage of the amphibian egg — an electron microscope study of the onset of cytokinesis in the egg of *Ambystoma mexicanum*. *J. Ultrastr. Res.* 32: 142-166.

BLUEMINK, J. G., 1971a. Cytokinesis and cytochalasin-induced furrow regression in the first-cleavage zygote of *Xenopus laevis*. *Z. Zellforsch.* 121: 102-126.

BLUEMINK, J. G., 1971b. Effects of cytochalasin B on surface contractility and cell junction formation during egg cleavage in *Xenopus laevis*. *Cytobiologie* 3: 176-187.

BLUEMINK, J. G., 1972. Cortical wound healing in the amphibian egg: an electron microscopical study. *J. Ultrastr. Res.* 41: 95-114.

BOCQUET-VÉDRINE, J. and J. POCHON-MASSON, 1969. Cytodifférentiation d'une vésicle de sécrétion au cours de la spermiogenèse chez *Balanus perforatus* Brug. (Crustacé Cirripède). *Archs Zool. exp. gén.* 110: 595-616.

BOOKHOUT, C. G. and J. D. COSTLOW, JR., 1959. Feeding, molting and growth in barnacles. Pages 212-225 in: *Marine Boring and Fouling Organisms* (D. L. Ray, ed.). Seattle: University of Washington Press.

BOUSFIELD, E. L., 1952-53. The distribution and spawning seasons of barnacles on the Atlantic coast of Canada. *Ann. Rep. Nat. Mus. Can.*, Bull. No. 132: 112-154.

BROCH, H., 1922. Studies on Pacific cirripedes: No. 10 in Papers from Dr. T. H. Mortensen's Pacific expedition 1914-1916. *Vidensk. Meddr. danske naturh.-Foren. Kbh.* 73: 215-358.

BROWN, G. G., 1966. Ultrastructural studies of sperm morphology and sperm-egg interaction in the decapod *Callinectes sapidus*. *J. Ultrastr. Res.* 14: 425-440.

BUCHHOLZ, H., 1951. Die larvenformen von *Balanus improvisus*. Beitrage zur kenntnis des larvenplanktons. *Kieler Meeresforsch.* 8: 49-57.

BUCKLEY, I. K., 1974. Subcellular motility: a correlated light and electron microscopic study using cultured cells. *Tissue & Cell* 6: 1-20.

BUCKLEY, I. K. and K. R. PORTER, 1967. Cytoplasmic fibrils in living cultured cells. A light and electron microscope study. *Protoplasma* 64: 349-380.

BURMEISTER, H., 1834. *Beiträg zur Naturgeschichte der Rankenfusser (Cirripedia). ser. 16.* Berlin: G. Reimer Pub. 60 pp.

BURNETT, B. R., 1972. Aspects of the circulatory system of *Pollicipes polymerus* J. B. Sowerby (Cirripedia: Thoracica). *J. Morph.* 136: 79-108.

BURNSIDE, B., 1971. Microtubules and microfilaments in newt neurulation. *Dev. Biol.* 26: 416-441.

BURNSIDE, B., 1973. Microtubules and microfilaments in amphibian neurulation. *Am. Zool.* 13: 989-1006.

BYERS, B. and D. H. ABRAMSON, 1968. Cytokinesis in HeLa: Post-telophase delay and microtubule-associated motility. *Protoplasma* 66: 413-435.

BYERS, B. and K. PORTER, 1964. Oriented microtubules in elongating cells of the developing lens rudiment after induction. *Proc. Nat. Acad. Sci. U.S.* 52: 1091-1099.

CARTER, S. B., 1967. Effects of cytochalasins on mammalian cells. *Nature* 213: 261-264.

CHEUNG, T. S., 1966. The development of egg-membranes and egg attachment in the shore crab *Carcinus maenas* and some related decapods. *J. Mar. Biol. Assoc.* 46: 373-400.

CHUN, C., 1896. Atlantis. Biologische studien uber pelagische organismen. Die nauplien der Lepaden (Nebst Bemerkungen uber das schwebvernogen der pelagisch lebenden Crustacean). *Bibl. Zool.* 7: 77-106.

CLAUS, C., 1869. Die cypris-ahnliche larve (Puppe) der cirripedien und ihre verwandlung in das fest-sitzende. *Thier. Marburg* 4-17.

CLEGG, D. J., 1957. Some observations on pairing in *Balanus balanoides*. *Rep. Challenger Soc.* 3: 18-19.

CLONEY, R. A., 1966. Cytoplasmic filaments and cell movements; epidermal cells during ascidian metamorphosis. *J. Ultrastr. Res.* 14: 300-328.

CLONEY, R. A., 1969. Cytoplasmic filaments and morphogenesis: the role of the notochord in ascidian metamorphosis. *Z. Zellforsch.* 100: 31-53.

CLONEY, R. A. and E. FLOREY, 1968. Ultrastructure of cephalopod chromatophore organs. *Z. Zellforsch.* 89: 250-280.

CLONEY, R. A., J. SCHAADT and J. V. DURDEN, 1970. Thermoelectric cooling stage for the compound microscope. *Acta zool.* 51: 95-98.

COCHRAN, W. G., 1947. Some consequences when the assumptions for the analysis of variance are not satisfied. *Biometrics* 3: 22-38.

COE, W. R., 1932. *Season of Attachment and Rate of Growth of Sedentary Marine Organisms at the Pier of the Scripps Institution of Oceanography, La Jolla, California.* Berkeley: U.C. Press. 86 pp.

COLLIER, A., S. RAY and W. B. WILSON, 1956. Some effects of specific organic compounds on marine organisms. *Science* 124: 220.

CONNELL, J. H., 1970. A predator-prey system in the marine intertidal region. I. *Balanus glandula* and several predatory species of *Thais*. *Ecol. Monogr.* 40: 49-78.

CONRAD, G. W., 1973. Control of polar lobe formation in fertilized eggs of *Ilyanassa obsoleta* Stimpson. *Am. Zool.* 13: 961-980.

CONRAD, G. W., 1974. Role of calcium ions in polar lobe formation by fertilized eggs of *Ilyanassa obsoleta*. *J. Cell Biol.* 63: 69a. (Abstract #138).

CONRAD, G. W., D. C. WILLIAMS, F. R. TURNER, K. M. NEWROCK and R. A. RAFF, 1973. Microfilaments in the polar lobe constriction of fertilized eggs of *Ilyanassa obsoleta*. *J. Cell Biol.* 59: 228-233.

CONRAD, G. W. and D. C. WILLIAMS, 1974a. Polar lobe formation and cytokinesis in fertilized eggs of *Ilyanassa obsoleta*. I. Ultrastructure and effects of cytochalasin B and colchicine. *Dev. Biol.* 36: 363-378.

CONRAD, G. W. and D. C. WILLIAMS, 1974b. Polar lobe formation and cytokinesis in fertilized eggs of *Ilyanassa obsoleta*. II. Large bleb formation caused by high concentrations of exogenous calcium ions. *Dev. Biol.* 37: 280-295.

CONTI, C. J. and A. J. P. KLEIN-SZANTO, 1973. Nuclear multivesicular bodies in cultured hamster cells. *Experientia* 29: 850-851.

CORNWALL, I. E., 1925. A review of the cirripedia of the coast of British Columbia with a glossary, and key to genera and species. *Contr. Can. Biol.* 2: 469-502.

CORNWALL, I. E., 1951. The barnacles of California (Cirripedia). *Wasmann J. Biol.* 9: 311-346.

CORNWALL, I. E., 1955a. The barnacles of British Columbia. British Columbia Provincial Museum, Handbook No. 7. 69 pp.

CORNWALL, I. E., 1955b. Arthropoda. 10e. Cirripedia. Ottawa: Can. Fisheries Res. Bd. Monogr. 49 pp.

COSTLOW, J. D., JR. and C. G. BOOKHOUT, 1953. Moulting and growth in *Balanus improvisus*. *Biol. Bull.* 105: 420-433.

COSTLOW, J. D., JR. and C. G. BOOKHOUT, 1957. Larval development of *Balanus eburneus* in the laboratory. *Biol. Bull.* 112: 313-324.

COSTLOW, J. D., JR. and C. G. BOOKHOUT, 1958. Larval development of *Balanus amphitrite* var. *denticulata* Broch reared in the laboratory. *Biol. Bull.* 114: 284-295.

CRISP, D. J., 1950. Breeding and distribution of *Chthamalus stellatus*. *Nature* 166: 311-312.

CRISP, D. J., 1953. Changes in orientation of barnacles of certain species in relation to water currents. *J. Anim. Ecol.* 22: 331-343.

CRISP, D. J., 1954. The breeding of *Balanus porcatus* (Da Costa) in the Irish Sea. *J. Mar. Biol. Assoc.* 33: 473-494.

CRISP, D. J., 1956. A substance promoting hatching and liberation of young in cirripedes. *Nature* 178: 263.

CRISP, D. J., 1957. Effect of low temperature on the breeding of marine animals. *Nature* 179: 1138-1139.

CRISP, D. J., 1958a. Breeding and exuviation in *Balanus balanoides*. *Proc. Fifteenth Internat. Congress of Zool.* Sect. 3, paper 7: 298-300.

CRISP, D. J., 1958b. Spread of *Elminius modestus* Darwin in Northwest Europe. *J. Mar. Biol. Assoc.* 37: 483-520.

CRISP, D. J., 1959a. Factors influencing the time of breeding of *Balanus balanoides*. *Oikos* 10: 275-289.

CRISP, D. J., 1959b. The rate of development of *Balanus balanoides* (L.) embryos *in vitro*. *J. Anim. Ecol.* 28: 119-132.

CRISP, D. J., 1960. Factors influencing growth-rate in *Balanus balanoides*. *J. Anim. Ecol.* 29: 95-116.

CRISP, D. J., 1962. The larval stages of *Balanus hameri* (Ascanius, 1767). *Crustaceana* 4: 123-130.

CRISP, D. J. and H. BARNES, 1954. The orientation and distribution of barnacles at settlement with particular reference to surface contour. *J. Anim. Ecol.* 23: 142-162.

CRISP, D. J. and P. CHIPPERFIELD, 1948. Occurrence of *Elminius modestus* in British waters. *Nature* 161: 64.

CRISP, D. J. and D. J. CLEGG, 1960. The induction of the breeding condition in *Balanus balanoides* (L.). *Oikos* 11: 265-275.

CRISP, D. J. and J. D. COSTLOW, JR., 1963. The tolerance of developing cirripede embryos to salinity and temperature. *Oikos* 14: 22-34.

CRISP, D. J. and P. A. DAVIES, 1955. Observations *in vivo* on the breeding of *Elminius modestus* grown on glass slides. *J. Mar. Biol. Assoc.* 34: 357-380.

CRISP, D. J. and P. S. MEADOWS, 1962. The chemical basis of gregariousness in cirripedes. *Proc. Roy. Soc. London* 156: 500-520.

CRISP, D. J. and P. S. MEADOWS, 1963. Adsorbed layers: the stimulus to settlement in barnacles. *Proc. Roy. Soc. London.* 158: 364-387.

CRISP, D. J. and B. PATEL, 1960. The moulting cycle in *Balanus balanoides* (L.). *Biol. Bull.* 118: 31-47.

CRISP, D. J. and B. PATEL, 1961. The interaction between breeding and growth rate in the barnacle *Elminius modestus* Darwin. *Limnol. Oceanogr.* 6: 105-115.

CRISP, D. J. and B. PATEL, 1969. Environmental control of the breeding of three boreo-arctic cirripedes. *Mar. Biol.* 2: 283-295.

CRISP, D. J. and A. J. SOUTHWARD, 1961. Different types of cirral activity of barnacles. *Phil. Trans. Roy. Soc. London* 243: 271-308.

CRISP, D. J. and C. P. SPENCER, 1958. The control of the hatching process in barnacles. *Proc. Roy. Soc. London* 149: 278-299.

CRISP, D. J. and H. G. STUBBINGS, 1957. The orientation of barnacles to water currents. *J. Anim. Ecol.* 26: 179-196.

CROWELL, J., 1964. The fine structure of the polar lobe of *Ilyanassa obsoleta*. *Acta Embryol. Morph. exp.* 7: 225-234.

CROZIER, W. J., 1916. On a barnacle, *Conchoderma virgatum*, attached to a fish, *Diodon hystrix*. *Amer. Nat.* 50: 637-639.

DABORN, G. R., 1974. Length-weight allometric relationships in four crustaceans from Alberta lakes and ponds. *Can. J. Zool.* 52: 1303-1310.

DANIEL, A., 1957. Illumination and its effect on the settlement of barnacle cyprids. *Proc. Zool. Soc. London* 129: 305-313.

DARWIN, C., 1851. A monograph on the subclass Cirripedia. I. Lepadidae. Ray Society, London. (No. 21).

DARWIN, C., 1854. A monograph on the subclass Cirripedia. II. Balanidae. Ray Society, London. (No. 25).

DAVSON, H., 1964. *A Textbook of General Physiology.* 3rd ed. Boston: Little, Brown & Co. 1166 pp.

DAWSON, R. M. C. and H. BARNES, 1966. Studies in the biochemistry of cirripede eggs. II. Changes in lipid composition during development of *Balanus balanoides* and *Balanus balanus.* *J. Mar. Biol.Assoc.* 46: 249-261.

DAYTON, P. K., 1971. Competition, disturbance and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecol. Monogr.* 41: 351-389.

DELAGE, Y., 1884. Evolution de la Sacculine (*Sacculina carcini*, Thomps.), crustacé endoparasite de l'ordre nouveau des kentrogonides. *Archs Zool. exp. gé*[illegible] 2: 417-736.

DELSMAN, H. C., 1917. Die embryonalentwicklung von *Balanus balanoides* Linn. *Helder Tydschr. Ned. Dierk. Ver.* 2: 419-520.

DeSANTO, R. S. and P. L. DUDLEY, 1969. Ultramicroscopic filaments in the ascidian *Botryllus schlosseri* (Pallas) and their possible role in ampullar contractions. *J. Ultrastr. Res.* 28: 259-274.

DOHMEN, M. R. and N. H. VERDONK, 1974. The structure of a morphogenetic cytoplasm, present in the polar lobe of *Bithynia tentaculata* (Gastropoda, Prosobranchia). *J. Embryol. exp. Morph.* 31: 423-433.

DONALDSON, S. and D. J. PRIOR, 1972. Endogenous activity in the central nervous system of the barnacle *Pollicipes polymerus.* Final Papers, Zool. 538. Friday Harbor Laboratories. (Unpublished).

DUDLEY, P. L., 1957. The development of the Notodelphid copepods and the application of larval characteristics to the systematics of some species from the Northeast Pacific. Doctoral Thesis, Univ. of Wash., Seattle, Wash.

DUDLEY, P. L., 1973. Synaptonemal polycomplexes in spermatocytes of the gooseneck barnacle, *Pollicipes polymerus* Sowerby (*Crustacea: Cirripedia*). *Chromosoma* 40: 221-242.

DUNLAP, HELEN L., 1966. Oogenesis in the Ctenophora. Doctoral Thesis, Univ. of Wash., Seattle, Wash.

DURHAM, A. C. H., 1974. A unified theory of the control of actin and myosin in nonmuscle movements. *Cell* 2: 123-135.

EASTMAN, R. C., 1968. Activities of several pentose shunt and glycolytic enzymes in developing eggs of the barnacle, *Pollicipes polymerus.* *Exp. Cell Res.* 51: 323-329.

EIGSTI, O. J., 1947. Colchicine bibliography. *Lloydia (Cincinnati)* 10: 65-114.

ELLIOTT, A. M., 1965. Primary lysosomes in *Tetrahymena pyriformis*. *Science* 149: 640-641.

EPEL, D., B. C. PRESSMAN, S. ELSAESSER and A. M. WEAVER, 1969. The program of structural and metabolic changes following fertilization of sea urchin eggs. Pages 279-298 in: *The Cell Cycle* (G. M. Padilla, G. L. Whitson and I. L. Cameron, eds.). New York: Academic Press.

EVANS, F., 1958. Growth and maturity of the barnacles *Lepas hillii* and *Lepas anatifera*. *Nature* 182: 1245-1246.

FAIRBRIDGE, R. W. (Ed.), 1966. Wave refraction. Pages 975-976 in: *The Encyclopedia of Oceanography*. Vol. 1. New York: Reinhold Pub. Corp.

FAWCETT, D. W., 1966. *An Atlas of Fine Structure*. Philadelphia: W. B. Saunders Co. 448 pp.

FREIBERGER, A. and C. P. COLOGER, 1966. Rearing acorn barnacles in the laboratory for marine fouling studies. *J. Am. Soc. Nav. Engrs*. 78: 881-890.

FREY-WYSSLING, A., 1953. *Submicroscopic Morphology of Protoplasm*. Amsterdam: Elsevier Pub. Co. 411 pp.

FYHN, H. J., J. A. PETERSEN and K. JOHANSEN, 1972. Eco-physiological studies of an intertidal crustacean, *Pollicipes polymerus* (Cirripedia, Lepadomorpha). I. Tolerance to body temperature change, desiccation and osmotic stress. *J. Exp. Biol.* 57: 83-102.

GANAPATHI, P. N., M. V. LAKSHMANA RAO and R. NAGABUSHANAM, 1958. Biology of fouling in the Visakhapatnam Harbor. *Andhra University Memoirs in Oceanography*. Ser. 62: 193-209.

GEORGE, M. J., 1958. Observations on the plankton of the Cochin backwaters. *Indian J. Fish.* 5: 375-401.

GÉRIN, Y., 1972. Morphogenèse des vésicules à double membrane du lobe polaire d'*Ilyanassa obsoleta* Say. Étude ultrastructurale. *J. Microscopie (Paris)* 13: 57-66.

GIBBINS, J. R., L. G. TILNEY and K. R. PORTER, 1969. Microtubules in the formation and development of the primary mesenchyme in *Arbacia punctulata*. I. The Distribution of microtubules. *J. Cell Biol.* 41: 201-226.

GIBSON, P. H. and J. A. NOTT, 1971. Concerning the fourth antennular segment of the cypris larva of *Balanus balanoides*. Pages 227-236 in: *Fourth European Marine Biological Symposium* (D. J. Crisp, ed.). Cambridge: Cambridge University Press.

GIESE, A., 1959. Comparative physiology: Annual reproductive cycles in marine invertebrates. *Ann. Rev. Physiol.* 21: 547-576.

GINGELL, D., 1970. Contractile responses at the surface of an amphibian egg. *J. Embryo. exp. Morph.* 23: 583-609.

GOLDMAN, R. D., G. BERG, A. BUSHNELL, C. M. CHANG, L. DICKERMAN, M. HOPKINS, M. L. MILLER, R. POLLACK and E. WANG, 1973. Fibrillar systems in cell mobility. Pages 83-107 in: *Ciba Foundation Symposium 14. Locomotion of Tissue Cells.* New York: Elsevier.

GOLDMAN, R. D. and E. A. C. FOLLETT, 1969. The structure of the major cell processes of isolated BHK21 fibroblasts. *Exp. Cell Res.* 57: 263-276.

GOODENOUGH, D. A., S. ITO and J.-P. REVEL, 1968. Electron microscopy of early cleavage stages in *Arbacia punctulata*. *Biol. Bull.* 135: 420-421.

GOODSIR, H. D. S., 1843. On the larvae in the first stage of development in *Balanus*. *Edinburgh New Phil. J.* 35: 88-104.

GRAY, J. E., 1833. On the reproduction of Cirripedia. *Proc. Zool. Soc. London* 1: 115-116.

GREEN, D. E. and H. BAUM, 1970. *Energy and the Mitochondrion.* New York: Academic Press. 205 pp.

GROOM, T. T., 1892. On the early development of cirripedia. *Phil. Trans. Roy. Soc. London* 52: 158-162.

GROOM, T. T., 1894. On the early development of cirripedia. *Phil. Trans. Roy. Soc. London* 185: 119-234.

GRUVEL, A., 1893. L'étude des cirripedes. *Archs Zool. exp. gén.* Ser. 3 1: 401-610.

GRUVEL, A., 1905. *Monographie des Cirripedes ou Thecostraces.* Paris: Masson & Cie.

GUILLARD, R. R. L. and J. H. RYTHER, 1962. Studies of marine planktonic diatoms. *Can. J. Microbiol.* 8: 229-239.

GULYAS, B. J., 1973. Cytokinesis in the rabbit zygote: fine structural study of the contractile ring and the mid-body. *Anat. Rec.* 177: 195-207.

GWILLIAM, C. F. and J. C. BRADBURY, 1971. Activity patterns in the isolated central nervous system of the barnacle and their relation to behavior. *Biol. Bull.* 141: 502-513.

HANCOCK, G. J., 1953. Self-propulsion of microscopic organisms through liquids. *Proc. Roy. Soc.* 217: 96-121.

HAND, C., J. D. STANDING and J. C. RUTHERFORD, 1973. San Francisco oil spill, effects on reproduction in intertidal invertebrates. Report for the Office of Research & Monitoring, Environmental Protection Agency. Project #15080.

HARA, K., 1971. Cinematographic observation of "surface contraction waves" (SCW) during the early cleavage of *Axolotl* eggs. *Wilhelm Roux' Archiv.* 167: 183-186.

HATTON, H., 1938. Essais de bionomie explicative sur quelques expèces intercotidales d'algues et d'animaux. *Ann. Inst. Ocean.* 17: 241-348.

HATTON, H. and E. FISCHER-PIETTE, 1932. Observations et experiences sur le peuplement des cotes rocheuses par les cirripèdes. *Bull. Inst. Ocean. Monaco.* No. 592: 1-15.

HENRY, D. P., 1940a. The cirripedia of Puget Sound with a key to the species. *Univ. Wash. Pub. Ocean.* 4: 1-48.

HENRY, D. P., 1940b. Notes on some pedunculate barnacles from the North Pacific. *Proc. U.S. Nat. Mus.* 88: 225-236.

HERZ, L. E., 1933. The culture and morphology of the later stages of *Balanus crenatus* Bruguiere. *Biol. Bull.* 64: 432-442.

HILDRETH, P. E., 1950. A study of the nauplius larvae of *Mitella polymerus*. Final Papers, Zool. 112-212. Bodega Bay Marine Station (Unpublished).

HILGARD, G. H., 1960. A study of reproduction in the intertidal barnacle *Mitella polymerus* in Monterey Bay, California. *Biol. Bull.* 119: 169-188.

HINKLEY, R. and A. TELSER, 1974. Heavy meromyosin-binding filaments in the mitotic apparatus of mammalian cells. *Exp. Cell Res.* 86: 161-164.

HINSCH, G. W. and M. V. CONE, 1969. Ultrastructural observations of vitellogenesis in the spider crab, *Libinia emarginata* L. *J. Cell Biol.* 40: 336-342.

HIRAMOTO, Y., 1958. A quantitative description of protoplasmic movement during cleavage in the sea urchin egg. *J. Exp. Biol.* 35: 407-424.

HIRANO, R., 1962. Mass rearing of barnacle larvae. *Bull. Mar. Biol. Station Asamushi* 11: 77-80.

HIRO, F., 1939. On the barnacle communities at the Madarai Pier in Kororu Island, Palao. *Palao Trop. Biol. Stn Stud.* 1: 585-595.

HOADLEY, L., 1934. Pulsations in the *Nereis* egg. *Biol. Bull.* 67: 484-493.

HOFFMAN-BERLING, H., 1958. Der mechanismus eines neuen, von der muskelkontraktion verschiedenen kontraktionszyklus. *Biochim. Biophys. Acta* 27: 247-255.

HOLSTROM, M. V., 1970. Distribution, reproduction, recruitment and pigments of the stalked barnacle *Pollicipes polymerus* in the vicinity of sewage outfalls at Pacific Grove and Carmel, California. Final papers, Biol. 175h. Hopkins Marine Station. (Unpublished).

HOLTER, A. R., 1969. Carotenoid pigments in the stalked barnacle *Pollicipes polymerus*. *Comp. Biochem. Physiol.* 28: 675-684.

HOLTZER, H. and J. W. SANGER, 1972. Cytochalasin B: Microfilaments, cell movement and what else? *Dev. Biol.* 27: 444-446.

HONIG, G. R. and M. RABINOVITZ, 1965. Actinomycin D: Inhibition of protein synthesis unrelated to effect on template RNA synthesis. *Science* 149: 1504-1506.

HOWARD, G. K., 1959. Aspects of the feeding and reproductive biology of *Mitella polymerus*. M.S. Thesis, Stanford University.

HOWARD, G. K. and H. C. SCOTT, 1959. Predaceous feeding in two common gooseneck barnacles. *Science* 129: 717-718.

HUTCHINS, L. W., 1947. The bases for temperature zonation in geographical distribution. *Ecol. Monogr.* 17: 325-335.

HUXLEY, H. E., 1973. Muscular contraction and cell motility. *Nature* 243: 445-449.

JAHN, T. L. and E. C. BOVEE, 1969. Protoplasmic movements within cells. *Physiol. Rev.* 49: 793-862.

JOHN, P. A., 1964. Biology of *Balanus amphitrite communis* (Darwin) in the Cochin Harbour water. *Fish. Technol.* 1: 189-201.

JOHNSON, M. W. and T. G. THOMPSON, 1929-31. The sea water at the Puget Sound Biological Station from September 1927 to September 1928. *Puget Sound Biol. Station Pubs.* 7: 119-128.

JONES, L. W. G. and D. J. CRISP, 1954. The larval stages of the barnacle *Balanus improvisus* Darwin. *Proc. Zool. Soc. London* 123: 765-780.

KALT, M. R., 1971. The relationship between cleavage and blastocoel formation in *Xenopus laevis*. II. Electron microscopic observations. *J. Embryol. exp. Morph.* 26: 51-66.

KAMIYA, N., 1960. Physics and chemistry of protoplasmic streaming. *Ann. rev. Plant Physiol.* 11: 323-340.

KANG, Y.-H., 1974. Development of the zona pellucida in the rat oocyte. *Am. J. Anat.* 139: 535-566.

KARANDE, A. A., 1974. Larval development of the barnacle *Tetraclitella karandei* reared in the laboratory. *Biol. Bull.* 146: 249-257.

KARASAKI, S., 1963. Studies on amphibian yolk. V. Electron microscopic observations on the utilization of yolk platelets during embryogenesis. *J. Ultrastr. Res.* 9: 225-247.

KARFUNKEL, P., 1971. The role of microtubules and microfilaments in neurulation in *Xenopus*. *Dev. Biol.* 25: 30-56.

KAUFMANN, R., 1965. Zur embryonal-und larvalentwicklung von *Scalpellum scalpellum* L. (Crustacea Cirripedia) mit einem beitrag zur autokologie dieser art. *Z. Morph. Okol. Tiere* 55: 161-232.

KENNELL, D., 1964. Persistence of messenger RNA activity in *Bacillus megaterium* treated with actinomycin. *J. Mol. Biol.* 9: 789-800.

KESSEL, R. G., 1966. Electron microscopic studies on the origin and maturation of yolk in oocytes of the tunicate *Ciona intestinales*. *Z. Zellforsch.* 71: 525-544.

KITCHING, J. A., 1950. Distribution of the littoral barnacle *Chthamalus stellatus* around the British Isles. *Nature* 165: 820.

KLETZIEN, R. F., J. F. PERDUE and A. SPRINGER, 1972. Cytochalasin A & B: Inhibition of sugar uptake in cultured cells. *J. Biol. Chem.* 247: 2964-2966.

KNIGHT-JONES, E. W., 1953. Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. *J. Exp. Biol.* 30: 584-598.

KNIGHT-JONES, E. W. and G. D. WAUGH, 1949. On the larval development of *Elminius modestus* Darwin. *J. Mar. Biol. Assoc.* 28: 413-438.

KOMNICK, H., W. STOCKEM and K. E. WOHLFARTH-BOTTERMANN, 1973. Cell mobility: mechanisms in protoplasmic streaming and amoeboid movement. *Inter. Rev. Cytol.* 34: 169-249.

KOSSMAN, R., 1874. Suctoria und Lepadidae. *Arb. Zoolog.-Zootom. Inst. Wurzberg.* 1: 179-207.

KRISHAN, A., 1971. Fine structure of cytochalasin-induced multinucleated cells. *J. Ultrastr. Res.* 36: 191-204.

KROHN, A., 1859. Beobachtungen uber den cementapparat und die weiblichen zeugungsorgane einiger cirripedien. *Arch. Naturgesch.* 25: 355-364.

KUHL, H., 1950. Uber die normale und die durch gifte beeinflusste metamorphose von *Balanus improvisus*. Herre. Verh. Deuts. Zoologen in Mainz. 1949. Leipzig. pp. 158-167.

KUZNETZOV, W. W. and T. A. MATVEEVA, 1949. The influence of the density of populations on certain biological processes in *Balanus balanoides* (L.) from Eastern Murman. *Rept. Acad. Sci. U.S.S.R.* 64: 413-415.

LANG, A., 1878. Ueber die metamorphosen der nauplius-larven von *Balanus*. *Mitt. d. aargau. Naturforsch. Gesell. (Aarau)*. 1: 104-115.

LASH, J., R. A. CLONEY and R. R. MINOR, 1970. Tail resorption in ascidians: Effect of cytochasin B. *Biol. Bull.* 139: 427-428.

LEHNINGER, A. L., 1964. *The Mitochondrion; Molecular Basis of Structure and Function*. New York: Benjamin. 263 pp.

LEWIS, C. A., 1975a. Some observations on factors affecting embryonic and larval growth of *Pollicipes polymerus* (Cirripedia: Lepadomorpha) *in vitro*. *Mar. Biol.* (In press).

LEWIS, C. A., 1975b. Development of the gooseneck barnacle *Pollicipes polymerus* (Cirripedia: Lepadomorpha): Fertilization through settlement. *Mar. Biol.* (In press).

LEWIS, C. A., F.-S. CHIA and T. E. SCHROEDER, 1973. Peristaltic constrictions in fertilized barnacle eggs (*Pollicipes polymerus*). *Experientia* 29: 1533-1535.

LOCKE, M. and J. V. COLLINS, 1967. Protein uptake in multivesicular bodies in the molt-intermolt cycle of an insect. *Science* 155: 467-469.

LOCKWOOD, A. P. M., 1967. *Aspects of the Physiology of Crustacea*. San Francisco: W. H. Freeman and Co. 328 pp.

LOOSANOFF, V. L., H. C. DAVIS and P. E. CHANLEY, 1953. Behavior of clam larvae in different concentrations of food organisms. *Anat. Rec.* 117: 586-587.

LUCKENS, P. A., 1968. The breeding and settlement of *Chthamalus challengeri* Hoek at Asamushi during 1967. *Bull. Biol. Station Asamushi* 8: 75-82.

LUCKS, R., 1940. Crustaceen und rotatorien aus den brackgewassern der Danziger umgebung. *Bericht des West preussischen Bot.-Zool. Vereins, Danzig* 62: 1-39.

LUFT, J. H., 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9: 409-414.

LUFT, J. H., 1971. Ruthenium red and violet II. Fine structure localization in animal tissues. *Anat. Rec.* 171: 369-392.

LUNDGREN, B., 1973. Surface coatings of the sea urchin larva, as revealed by Ruthenium Red. *J. Submicroscopic Cytol.* 5: 61-67.

McGUIRE, J. and G. MOELLMANN, 1972. Cytochalasin B: Effects on microfilaments and movement of melanin granules within melanocytes. *Science* 175: 642-644.

MALAWISTA, S. E., H. SATO and K. G. BENSH, 1968. Vinblastine and griseofulvin reversibly disrupt the living mitotic spindle. *Science* 160: 770-772.

MALECH, H. L. and T. L. LENTZ, 1974. Microfilaments in epidermal cancer cells. *J. Cell Biol.* 60: 473-482.

MEKKER, H. J. and B. ZIMMERMANN, 1970. Electron microscopic studies on the oocyte maturation in cultures of juvenile rat ovaries. *Z. Zellforsch.* 111: 364-378.

MENGE, B. A., 1974. Effect of wave action and competition on brooding and reproductive effort in the seastar, *Leptasterias hexactis*. *Ecology* 55: 84-93.

MILEIKOVSKII, S. A., 1958. Lunar periodicity in spawning of littoral and upper-sublittoral invertebrates of the White Sea and other seas. *Akademiia Nauk S.S.S.R. Doklady - Biol. Sci.* v. 120: 1006-1009.

MILLONIG, G., 1961. Advantages of a phosphate buffer for osmium tetroxide solutions in fixation. *J. Applied Physics* 32: 1637.

MIRANDA, A. F., G. C. GODMAN and S. W. TANENBAUM, 1974. Action of cytochalasin D on cells of established lines. II. Cortex and microfilaments. *J. Cell Biol.* 62: 406-423.

MIZEL, S. B. and L. WILSON, 1972. Inhibition of the transport of several hexoses in mammalian cells by cytochalsin B. *J. Biol. Chem.* 247: 4102-4105.

MOLENOCK, J. and E. D. GOMEZ, 1972. Larval stages and settlement of the barnacle *Balanus* (Conopea) *galeatus* (L.) (Cirripedia Thoracica). *Crustaceana* 23: 100-108.

MOORE, A. R., 1933. Is cleavage rate a function of the cytoplasm or of the nucleus? *J. Exp. Biol.* 10: 230-236.

MOORE, H. B., 1933. Change of orientation of a barnacle after metamorphosis. *Nature* 132: 969-970.

MOORE, H. B., 1934a. The biology of *Balanus balanoides*. I. Growth rate and its relation to size, season and tidal level. *J. Mar. Biol. Assoc.* 19: 851-868.

MOORE, H. B., 1934b. The growth rate of *Balanus*. *Scot. Nat.* 208: 101-109.

MOORE, H. B., 1935a. The biology of *Balanus balanoides*. III. The soft parts. *J. Mar. Biol. Assoc.* 20: 263-277.

MOORE, H. B., 1935b. The biology of *Balanus balanoides*. IV. Relation to environmental factors. *J. Mar. Biol. Assoc.* 20: 279-307.

MOORE, H. B. and J. A. KITCHING, 1939. The biology of *Chthamalus stellatus* (Poli). *J. Mar. Biol. Assoc.* 23: 521-541.

MORRILL, G. A., A. B. KOSTELLOW and J. B. MURPHY, 1971. Sequential forms of ATP as activity correlated with changes in cation binding and membrane potential from meiosis to first cleavage in *Rana pipiens*. *Exp. Cell Res.* 66: 289-298.

MORRILL, J. B. and F. O. PERKINS, 1973. Microtubules in the cortical region of the egg of *Lymnaea* during cortical segregation. *Dev. Biol.* 33: 206-212.

MORTLOCK, A. M., 1969. One approach to the barnacle problem. *J. Roy. Nav. S. S.* 24: 260-270.

MOYSE, J., 1960. Mass rearing of barnacle cyprids in the laboratory. *Nature* 185: 120.

MOYSE, J., 1961. The larval stages of *Acasta spongites* and *Pyrgoma anglicum* (Cirripedia). *Proc. Zool. Soc. London* 137: 371-392.

MOYSE, J., 1963. A comparison of the value of various flagellates and diatoms as food for barnacle larvae. *Inter. Council Explor. Sea* 28: 175-187.

MOYSE, J. and E. W. KNIGHT-JONES, 1967. Biology of cirripede larvae. Pages 595-611 in: *Proceedings of the Symposium on Crustacea.* Ernakulam: Mar. Biol. Assoc. India Symposium, ser. 2.

MÜLLER, F., 1868. On *Balanus armatus* and a hybrid between this species and *Balanus improvisus*, var. *assimilis*, Darw. *Ann. Mag. Nat. Hist.* Ser. 4: 393-412.

MUNN, E. A. and H. BARNES, 1970a. The structure of the axial filament complex of the spermatozoa of *Balanus balanus*. *Exp. Cell Res.* 60: 277-284.

MUNN, E. A. and H. BARNES, 1970b. The fine structure of the spermatozoa of some cirripedes. *J. Exp. Mar. Biol. Ecol.* 4: 261-286.

MUNTER, J. and R. BUCHHOLTZ, 1869. Uber *Balanus improvisus*. *Mittheilungen aus dem Naturwissenschaften, Verein von Neu-Vorpommern und Rügen.* 1: 1-40.

NAGAI, R. and L. I. REBHUN, 1966. Cytoplasmic microfilaments in streaming *Nitella* cells. *J. Ultrastr. Res.* 14: 571-589.

NASSONOW, N. B., 1885. Zur embryonalentwicklung von *Balanus*. *Zool. Anz.* 8: 44-46.

NASSONOW, N. B., 1887. Zur entwicklungsgeschichte der krebsformen *Balanus* und *Artemia*. *Isvest. Moscow Univ.* 52: 1-14.

NEWCOMBE, C. L., 1935. A study of the community relationships of the sea mussel, *Mytilus edulis* L. *Ecology* 16: 234-243.

NEWMAN, W. A., 1965. Prospectus on larval cirripede setation formulae. *Crustaceana* 9: 51-56.

NILSSON-CANTELL, C. A., 1921. Cirripedien studien. Zur kenntnis der biologie, anatomie und systematik dieser Gruppe. *Zool. Bidr. Uppsala* 7: 75-395.

NORREVANG, A., 1966. The ultrastructure of oocyte growth. Page 659 in: *The 6th International Congress for Electron Microscopy.* (Ryozi. Uyeda, ed.). Tokyo: Maruzen Co. Ltd.

NORREVANG, A., 1968. Electron microscopic morphology of oogenesis. *Internat. Rev. Cytol.* 23: 113-186.

NORRIS, E. and D. J. CRISP, 1953. The distribution and planktonic stages of the cirripede *Balanus perforatus* (Bruguière). *Proc. Zool. Soc. London* 123: 393-409.

NORRIS, E., L. W. JONES, T. LOVEGROVE and D. J. CRISP, 1951. Variability in larval stages of cirripedes. *Nature* 167: 444-445.

NOTT, J. A., 1969. Settlement of barnacle larvae: surface structure of the antennular attachment disc by scanning electron microscopy. *Mar. Biol.* 2: 248-251.

NOTT, J. A. and B. A. FOSTER, 1969. On the structure of the antennular attachment organ of the cypris larva of *Balanus balanoides* (L.). *Phil. Trans. Roy. Soc. London* 256: 115-134.

NUSSBAUM, M., 1890. *Anatomische Studien an Californischen Cirripedien.* Bonn, Germany: M. Cohen and Sohn (Pub.)

O'BRIEN, T. P. and K. V. THIMANN, 1966. Intracellular fibers in oat coleoptile cells and their possible significance in cytoplasmic streaming. *Proc. Nat. Acad. Sci. U.S.* 56: 888-894.

OKAMOTO, E., 1967. Utilization of *Platymonas*, *Nitzchia*, *Dunaliella*, *Chlorella* and *Stichococcus* in rearing larvae of the barnacles *Balanus glandula* and *Chthamalus dalli*. Final Papers, Biol. 175h. Hopkins Marine Station. (Unpublished).

ORCI, L. and W. STAUFFACHER, 1971. Glycogenosomes in renal tubular cells of diabetic animals. *J. Ultrastr. Res.* 36: 499-503.

ORCI, L., K. H. GABBAY and W. J. MALAISSE, 1972. Pancreatic beta-cell web: Its possible role in insulin secretion. *Science* 175: 1128-1130.

ORTON, J. H., 1920. Sea temperature, breeding and distribution in marine animals. *J. Mar. Biol. Assoc.* 12: 339-366.

PAINE, R. T., 1974. Intertidal community structure. Experimental studies on the relationship between a dominant competitor and its principal predator. *Oecologia* 15: 93-120.

PALADE, G. E., 1952. A study of fixation for electron microscopy. *J. Exptl. Med.* 95: 285-298.

PASTAN, I. and R. M. FRIEDMAN, 1968. Actinomycin D: Inhibition of phospholipid synthesis in chick embryo cells. *Science* 160: 316-317.

PASTEELS, J. J., 1949. Mouvements localisés et rythmiques de la membrane de fécondation chez les oeufs fécondés et activés (*Chaetopterus, Mactra, Nereis*). *Arch. Biol. (Liège)* 61: 199-220.

PASTEELS, J. J., 1966. The cortical movements of *Barnea candida* (bivalve mollusc) egg studied with an electron microscope. *J. Embryol. exp. Morph.* 16: 311-320.

PASTEELS, J. J. and E. de HARVEN, 1963. Etude au microscope électronique du cytoplasme de l'oeuf vierge et fécondé de *Barnea candida* (Mollusque bivalve). *Arch. Biol. (Liege)* 74: 415-437.

PATEL, B. S., 1959. The influence of temperature on the reproduction and moulting of *Lepas anatifera* (L.) under laboratory conditions. *J. Mar. Biol. Assoc.* 38: 589-597.

PATEL, B. S. and D. J. CRISP, 1960a. The influence of temperature on the breeding and the moulting activities of some warm-water species of operculate barnacles. *J. Mar. Biol. Assoc.* 39: 667-680.

PATEL, B. S. and D. J. CRISP, 1960b. Rates of development of the embryos of several species of barnacles. *Physiol. Zool.* 33: 104-119.

PEARSE, J. S., 1970. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. III. The echinoid *Diadema setosum* (Leske). *Bull. Mar. Sci.* 20: 697-720.

PERDUE, J. F., 1973. The distribution, ultrastructure and chemistry of microfilaments in cultured chick embryo fibroblasts. *J. Cell Biol.* 58: 265-283.

PERKINS, E. J., 1956. Preparation of copepod mounts for taxonomic work and for permanent collections. *Nature* 178: 1075-1076.

PERRY, M. M., 1967. Identification of glycogen in thin sections of amphibian embryos. *J. Cell. Sci.* 2: 257-264.

PERRY, M. M. and C. H. WADDINGTON, 1966. Ultrastructure of blastopore cells in the newt. *J. Embryol. exp. Morph.* 15: 317-330.

PERRYMAN, E. K., 1969. *Procambarus simulans*: light-induced changes in neurosecretory cells and in ovarian cycle. *Trans. Am. Microsc. Soc.* 88: 514-524.

PESTKA, S., 1971. Inhibitors of ribosome functions. *Ann. Rev. Microbiol.* 25: 487-562.

PETERSEN, J. A., H. J. FYHN and K. JOHANSEN, 1974. Eco-physiological studies of an intertidal crustacean, *Pollicipes polymerus* (Cirripedia, Lepadomorpha): aquatic and aerial respiration. *J. Exp. Biol.* 61: 309-320.

PICKETT-HEAPS, J. D., 1967. Ultrastructure and differentiation in *Chara* sp. I. Vegetative cells. *Aust. J. Biol. Sci.* 20: 539-551.

PILLAI, N. K., 1958. Development of *Balanus amphitrite* with a note on the early larvae of *Chelonibia testudinaria*. *Bull. Res. Inst. Univ. Kerala* 60: 117-130.

PILSBRY, H. A., 1907. The barnacles (Cirripedia) contained in the collections of the U.S. Nat. Mus. The Lepadidae. *Bull. U.S. National Museum* No. 60: 1-122.

PILSBRY, H. A., 1916. The sessile barnacles (Cirripedia) contained in the collections of the U.S. National Museum; including monograph of the American species. *Bull. U.S. National Museum* No. 93: 1-366.

PILSBRY, H. A., 1921. Barnacles of the San Juan Islands, Washington. *Proc. U.S. Nat. Mus.* 59: 111-115.

PLAGEMANN, P. G. and R. D. ESTENSEN, 1972. Cytochalasin B. VI. Competitive inhibition of nucleoside transport by cultured Novikoff rat hepatoma cells. *J. Cell Biol.* 55: 179-185.

POLLARD, T. D. and R. R. WEIHING, 1974. Actin and myosin and cell movement. *CRC Crit. Rev. Biochem.* 2: 1-65.

POMERAT, C. M. and E. R. REINER, 1942. The influence of surface angle and of light in the attachment of barnacles and of other sedentary organisms. *Biol. Bull.* 82: 14-25.

POMERAT, C. M. and C. M. WEISS, 1946. The influence of texture and composition of surface in the attachment of sedentary marine organisms. *Biol. Bull.* 91: 57-65.

PORTER, K. R., 1973. Microtubules in intracellular locomotion. Pages 149-169 in: *Ciba Foundation Symposium 14, Locomotion of Tissue Cells*. New York: Elsevier.

PUCCI-MINAFRA, I., S. MINAFRA and J. R. COLLIER, 1969. Distribution of ribosomes in the egg of *Ilyanassa obsoleta*. *Exp. Cell Res.* 57: 167-178.

PYEFINCH, K. A., 1948a. Methods of identification of the larvae of *Balanus balanoides* (L.), *Balanus crenatus* Bruguière and *Verruca stroemia* O. F. Müller. *J. Mar. Biol. Assoc.* 27: 451-463.

PYEFINCH, K. A., 1948b. Notes on the biology of cirripedes. *J. Mar. Biol. Assoc.* 27: 464-503.

PYEFINCH, K. A., 1949. The larval stages of *Balanus crenatus* Bruguière. *Proc. Zool. Soc. London* 118: 916-923.

RAFFIN, T., 1967. RNA synthesis during embryogenesis and later development in the barnacle *Pollicipes polymerus*. Final Papers, Biol. 175h, Hopkins Marine Station. (Unpublished).

RAPPAPORT, R., JR., 1960. The origin and formation of blastoderm cells of gammarid crustacea. *J. Exp. Zool.* 144: 43-59.

REAVEN, E. P. and S. G. AXLINE, 1973. Subplasmalemmal microfilaments and microtubules in resting and phagocytizing cultivated macrophages. *J. Cell Biol.* 59: 12-27.

REBHUN, L. I., 1967. Structural aspects of saltatory particle movement. *J. Gen. Physiol., Symp. Proc.* 50: 223-239.

REVERBERI, G., 1970. The ultrastructure of *Dentalium* egg at the trefoil stage. *Acta Embryol. Morph. exp.* 1: 31-43.

REYNOLDS, E. S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17: 208-212.

RICE, L., 1930. Peculiarities in the distribution of barnacles in communities and their probable causes. *Puget Sound Biol. Station Pubs.* 7: 249-257.

RICHARDSON, K. C., L. JARRETT and E. H. FINKE, 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Tech.* 35: 313-323.

RICKETTS, E. F. and J. CALVIN, 1968. *Between Pacific Tides*. 4th ed. (J. Hedgpeth, ed.). Stanford, Calif.: Stanford University Press. 614 pp.

ROBBINS, E. and N. K. GONATAS, 1964a. Histochemical and ultrastructural studies on HeLa cell cultures exposed to spindle inhibitors with special reference to the interphase cell. *J. Histochem. Cytochem.* 12: 704-711.

ROBBINS, E. and N. K. GONATAS, 1964b. Ultrastructure of a mammalian cell during the mitotic cycle. *J. Cell Biol.* 21: 429-463.

RODEGKER, W. and J. C. NEVENZEL, 1964. The fatty acid composition of three marine invertebrates. *Comp. Biochem. Physiol.* 11: 53-60.

ROODYN, D. B. (Ed.), 1967. *Enzyme Cytology*. London and New York: Academic Press. 587 pp.

ROSENBERG, M. D., 1967. Single cell properties—membrane development. Pages 18-38 in: *Ciba Foundation Cell Symposium. Differentiation*. (A.V.S. de Reuck and J. Knight, eds.). Boston: Little Brown.

ROTH, T. F. and K. R. PORTER, 1964. Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti* L. *J. Cell Biol.* 20: 313-332.

RUNNSTROM, S., 1925. Zur biologie und entwicklung von *Balanus balanoides* (Linne). *Bergens Mus. Aarbok, Naturv. Raekke* 5: 1-46.

RUNNSTROM, S., 1927. Uber die thermopathie der fortpflanzung und entwicklung mariner. Tiere in Beziehung zu irher geographischen Verbreitung. *Bergens Mus. Aarbok, Naturv. Raekke* 2: 1-67.

SAINT ANGE, M., 1835. Memoire sur l'organisation des cirripèdes. *Roy. Acad. Sci. Memoir* 6: 513-555.

SANDISON, E. E., 1954. The identification of the nauplii of some South African barnacles with notes on their life histories. *Trans. Roy. Soc. S. Afr.* 34: 69-101.

SANDISON, E. E., 1966. The effect of salinity fluctuations on the life cycle of *Balanus pallidus stutsburi* Darwin in Lagos Harbour, Nigeria. *J. Anim. Ecol.* 35: 363-378.

SANDISON, E. E., 1967. The naupliar stages of *Balanus pallidus stutsburi* Darwin and *Chthamalus aestuarii* Stubbings (Cirripedia Thoracica). *Crustaceana* 13: 161-174.

SANGER, J. W. and H. HOLTZER, 1972. Cytochalasin B: Effects on cell morphology, cell adhesion, and mucopolysaccharide synthesis. *Proc. Nat. Acad. Sci. U.S.* 69: 253-257.

SAWAI, T. and M. YONEDA, 1974. Wave of stiffness propagating along the surface of the newt egg during cleavage. *J. Cell Biol.* 60: 1-7.

SCHJEIDE, O. A., R. I.-S. LIN and R. J. MUNN, 1966. Isolation, composition and syntheses of multivesicular bodies. *J. Cell Biol.* 31: 100A.

SCHROEDER, T. E., 1968. Cytokinesis: filaments in the cleavage furrow. *Exp. Cell Res.* 53: 272-276.

SCHROEDER, T. E., 1969. The role of "contractile ring" filaments in dividing *Arbacia* eggs. *Biol. Bull.* 137: 413-414.

SCHROEDER, T. E., 1970a. Neurulation in *Xenopus laevis*. An analysis and model based upon light and electron microscopy. *J. Embryol. exp. Morph.* 23: 427-462.

SCHROEDER, T. E., 1970b. The contractile ring: I. Fine structure of dividing mammalian (HeLa) cells and the effects of cytochalasin B. *Z. Zellforsch.* 109: 431-449.

SCHROEDER, T. E., 1971. Mechanisms of morphogenesis: the embryonic neural tube. *Inter. J. Neuroscience* 2: 183-198.

SCHROEDER, T. E., 1972. The contractile ring. II. Determining its brief existence, volumetric changes, and vital role in cleaving *Arbacia* eggs. *J. Cell Biol.* 53: 419-434.

SCHROEDER, T. E., 1973a. Actin in dividing cells: contractile ring filaments bind heavy meromyosin. *Proc. Nat. Acad. Sci. U.S.* 70: 1688-1692.

SCHROEDER, T. E., 1973b. Cell constriction: contractile role of microfilaments in division and development. *Am. Zool.* 13: 949-960.

SCHROEDER, T. E. and D. L. STRICKLAND, 1974. Ionophore A23187, calcium and contractility in frog eggs. *Exp. Cell Res.* 3: 139-142.

SELMAN, G. G. and M. M. PERRY, 1970. Ultrastructural changes in the surface layers of the newt's egg in relation to the mechanism of its cleavage. *J. Cell Sci.* 6: 202-227.

SENECHAL, E., 1969. Observations on the feeding responses of the stalked barnacle *Pollicipes (Mitella) polymerus*. Final Papers, Zool. 157. Bodega Bay Marine Station. (Unpublished).

SHELFORD, V. E., 1930. Geographic extent and succession in Pacific North American intertidal *(Balanus)* communities. *Puget Sound Biol. Station Pubs.* 7: 217-223.

SHELFORD, V. E. and E. D. TOWLER, 1925. Animal communities of the San Juan Channel and adjacent areas. *Puget Sound Pubs.* 5: 33-73.

SINGH, A., 1974. The subplasmalemmal microfilaments in Kupffer cells. *J. ultrastr. Res.* 48: 67-69.

SKERMAN, T. M., 1958. Rates of growth in two species of *Lepas* (Cirripedia). *New Zealand J. Sci.* 1: 402-411.

SNODGRASS, J. B., 1967. Studies on the larval stages of the barnacles *Balanus glandula* Darwin, 1854, *Balanus tintinnabulum* (Linneus, 1758), *Chthamalus dalli* Pilsbry, 1916, and *Pollicipes polymerus* (Sowerby, 1833). Final papers, Biol. 175h, Hopkins Marine Station. (Unpublished).

SOKAL, R. R. and F. ROHLF, 1969. *Biometry.* San Francisco: Freeman. 776 pp.

SOUTHWARD, A. J., 1950. Occurrence of *Chthamalus stellatus* in the Isle of Man. *Nature* 165: 410-411.

SOUTHWARD, A. J., 1955a. On the behavior of barnacles. I. The relation of cirral and other activities to temperature. *J. Mar. Biol. Assoc.* 34: 403-422.

SOUTHWARD, A. J., 1955b. On the behavior of barnacles. II. The influence of habitat and tide-level on cirral activity. *J. Mar. Biol. Assoc.* 34: 423-433.

SOUTHWARD, A. J., 1957. On the behavior of barnacles. III. Further observations on the influence of temperature and age on cirral activity. *J. Mar. Biol. Assoc.* 36: 323-334.

SOUTHWARD, A. J. and D. J. CRISP, 1956. Fluctuations in the distributions and abundance of intertidal barnacles. *J. Mar. Biol. Assoc.* 35: 211-229.

SOUTHWARD, A. J. and D. J. CRISP, 1958. Modes of cirral activity in barnacles. *Proc. 15th Inter. Congress Zool.* Sect. III, paper No. 5.

SOUTHWARD, A. J. and D. J. CRISP, 1965. Activity rhythms of barnacles in relation to respiration and feeding. *J. Mar. Biol. Assoc.* 45: 161-185.

SOWERBY, G. B., 1833. [No title]. *Proc. Zool. Soc. London.* p. 74.

SPENCE-BATE, C., 1851. On the development of the cirripedia. *Ann. Mag. Nat. Hist.* Second series 8: 324-332.

SPOONER, B. S., J. F. ASH, J. T. WRENN, R. B. FRATER and N. K. WESSELLS, 1973. Heavy mesomyosin binding to microfilaments involved in cell and morphogenetic movements. *Tissue & Cell* 5: 37-46.

SPOONER, B. S., K. M. YAMADA and N. K. WESSELS, 1971. Microfilaments and cell locomotion. *J. Cell Biol.* 49: 595-613.

STEELE, R. G. D. and J. H. TORRIE, 1960. *Principles and Procedures of Statistics with Special Reference to the Biological Sciences.* New York: McGraw-Hill Book Co., Inc.

STRAUGHAN, D., 1971. Breeding and larval settlement of certain intertidal invertebrates in the Santa Barbara Channel following pollution by oil. Pages 223-244 in: *Biological and Oceanographical Survey of the Santa Barbara Channel Oil Spill 1969-70.* Vol. 1. *Biology and Bacteriology* (Allan Hancock Foundation, Univ. Southern Calif.)

STRAUSS, F., 1819. [No title]. *Memoirs du Mus. d'Histoire Nat.* 5: 381.

SZOLLOSI, D., 1968. The contractile ring and changes of the cell surface during cleavage. *J. Cell Biol.* 39: 133A.

SZOLLOSI, D., 1970. Cortical cytoplasmic filaments of cleaving eggs: a structural element corresponding to the contractile ring. *J. Cell Biol.* 44: 192-209.

SZUBINSKA, B., 1971. "New membrane" formation in *Amoeba proteus* upon injury of individual cells, electron microscope observations. *J. Cell Biol.* 49: 747-772.

TARASOV, N. E. and G. B. ZEVINA, 1957. Cirripedia thoracica of the seas of the U.S.S.R. *Fauna Rossii* No. 69: 267 pp.

TENGSTRAND, G., 1931. *Balanus improvisus*, nagot om dess förekomst i Göta älva mynning. *Fauna och Flora* 26: 108-112.

THOMPSON, J. V., 1830. On the cirripedes or barnacles, Cork. *Zoological Researches.* Vol. 1 pt. 1 Memoir 4.

THOMPSON, J. V., 1835. Discovery of the metamorphosis in the second type of the cirripedes, viz. the Lepades, completing the natural history of these singular animals and confirming their affinity with the Crustacea. *Phil. Trans. Roy. Soc. London* Part II: 355-358.

THOMPSON, T. G. and L. D. PHIFER, 1937-8. Seasonal variations in the surface waters of San Juan Channel during the five year period, January 1931 to December 30, 1935. *J. Mar. Res.* 1: 34-59.

TIGHE-FORD, D. J., 1967. Possible mechanism for the endocrine control of breeding in a cirripede. *Nature* 216: 920-921.

TIGHE-FORD, D. J., M. J. D. POWER and D. C. VAILE, 1970. Laboratory rearing of barnacle larvae for antifouling research. *Helgolander wiss. Meeresunters.* 20: 393-405.

TILNEY, L. G., 1969. Microtubules and filaments in the filopodia of the secondary mesemchyme cells of *Arbacia punctulata* and *Echinarachnius parma.* *J. Cell Sci.* 5: 195-210.

TILNEY, L. G. and R. R. CARDELL, Jr., 1970. Factors controlling the reassembly of the microvillus border of the small intestine of the salamander. *J. Cell Biol.* 47: 408-422.

TILNEY, L. G. and D. MARSLAND, 1969. A fine structural analysis of cleavage induction and furrowing in the eggs of *Arbacia punctulata. J. Cell Biol.* 42: 170-184.

TIMOURIAN, H., M. M. JOTZ and G. E. CLOTHIER, 1974. Intracellular distribution of calcium and phosphorus during the first cell division of the sea urchin egg. *Exp. Cell Res.* (In press).

TOWLER, E. D., 1930. An analysis of the intertidal barnacle communities of the San Juan Archipelago. *Puget Sound Biol. Station Pubs.* 7: 225-232.

TREAT, D. A., 1937. A comparative study of barnacle larvae. M.A. Thesis. Western Reserve University. (Unpublished).

TRICKER, R. A. R., 1965. *Bores, Breakers, Waves and Wakes. An Introduction to the Study of Waves on Water.* New York: American Elsevier Publishing Co., Inc.

TUCKER, J. B., 1971. Microtubules and a contractile ring of microfilaments associated with a cleavage furrow. *J. Cell Sci.* 8: 557-571.

TURQUIER, Y. and J. POCHON-MASSON, 1969. Spermatozoan infrastructure in *Trypetesa* (=Alcippe) *nassarioides* Turquier (Acrothoracica Cirripedia). *Archs Zool. exp. gén.* 110: 453-470.

U.S. NAVAL HYDROGRAPHIC OFFICE, 1943. World Atlas of Sea Surface Temperatures. H. O. No. 225.

van BENEDEN, E., 1870. Embryology recherches sur l'embryogenie des crustaces. III. Developpement de l'oeuf et de l'embryon des Sacculines (*Sacculini carcini*, Thomps.). *Bull. Acad. Roy. Belgique* ser. 2. 29: 99-112.

van BREEMEN, L., 1934. Zur biologie von *Balanus improvisus* (Darwin). *Zool. Anz. Leipzig* 105: 247-257.

van HOEK, P. P. C., 1876. Zur entwicklungsgeschichte der entomostraken. I. Embryologie von *Balanus. Niederl. Arch. Zool.* 3: 47-83.

van HOEK, P. P. C., 1884. Cirripedia collected by HMS *Challenger* during the years 1873-76. U.S. National Museum, *Challenger Report. Zoology* 10: 1-47.

van HOEK, P. P. C., 1909. Die cirripedien des Nordischen planktons. *Zoology* 4: 265-331.

VERDONK, N. H., W. L. M. GEILENKIRCHEN and L. P. M. TIMMERMANS, 1971. The localization of morphogenetic factors in uncleaved eggs of *Dentalium. J. Embryol. exp. Morph.* 25: 57-63.

VISSCHER, J. P., 1928. Reactions of the cyprid larvae of barnacles at the time of attachment. *Biol. Bull.* 54: 327-335.

VOLLMAR, H., 1972. Die einrollbewegung (Anatrepsis) des Keimstreifs im Ei von *Acheta domesticus* (Orthopteroidea, Gryllidae). *Wilhelm Roux' Archiv* 170: 135-151.

von WILLEMOES-SUHM, R., 1876. On the development of *Lepas fascicularis* and the Archizoea of cirripedia. *Phil. Trans. Roy. Soc. London* 166: 131-154.

WAGNER, R., 1834. Müllers Archiv fur Anatomie, Physiologie und Wissenschaftlichen Medicin. pp. 467-473.

WALLEY, L. J., 1965. The development and function of the oviducal gland in *Balanus balanoides*. *J. Mar. Biol. Assoc.* 45: 115-128.

WALLEY, L. J., 1969. Studies on the larval structure and metamorphosis of *Balanus balanoides* (L.). *Phil. Trans. Roy. Soc. London* 256: 237-280.

WALLEY, L. J., F. WHITE and K. M. BRANDER, 1971. Sperm activation and fertilization in *Balanus balanoides*. *J. Mar. Biol. Assoc.* 51: 489-494.

WARREN, R. H., 1968. The effect of colchicine on myogenesis *in vivo* in *Rana pipiens* and *Rhodnius prolixus* (Hemiptera). *J. Cell Biol.* 39: 544-555.

WEISENBERG, R. C., G. G. BORISY and E. W. TAYLOR, 1968. The colchicine-binding protein of mammalian brain and its relation to microtubules. *Biochem.* 7: 4466-4479.

WERNER, W. E., 1967. The distribution and ecology of the barnacle *Balanus trigonus*. *Bull. Mar. Sci.* 17: 64-84.

WESSELLS, N. K., B. S. SPOONER, J. F. ASH, M. O. BRADLEY, M. A. LUDUEÑA, E. L. TAYLOR, J. T. WRENN and K. M. YAMADA, 1971. Microfilaments in cellular and developmental processes. *Science* 171: 135-143.

WESSELLS, N. K., B. S. SPOONER and M. A. LUDUEÑA, 1973. Surface movements, microfilaments and cell locomotion. Pages 53-82 in: *Ciba Foundation Symposium 14. Locomotion of Tissue Cells*. New York: Elsevier.

WILLIAMS, E. J., 1959. *Regression Analysis*. New York: John Wiley & Sons. 214 pp.

WILLIAMS, G., 1950. Distribution of *Chthamalus stellatus* on the shores of north-east Ireland. *Nature* 166: 311.

WILLIAMSON, R. E., 1972. A light microscope study of the action of cytochalasin B on the cells and isolated cytoplasm of the Characeae. *J. Cell Sci.* 10: 811-819.

WINBERG, G. G., 1971. Growth, rate of development and fecundity in relation to environmental conditions. Pages 33-64 in: *Methods for the Estimation of Production of Aquatic Animals.* (G. G. Winberg, ed.). New York: Academic Press.

WISCHNITZER, S., 1970. An electron microscope study of cytoplasmic organelle transformation in developing mouse oocytes. *Wilhelm Roux' Archiv.* 166: 150-172.

WISELY, B., 1960. Experiments on rearing the barnacle *Elminius modestus* Darwin to the settling stage in the laboratory. *Aust. J. Mar. Freshwater Res.* 2: 42-54.

WOLF, D. P., 1974. The cortical response in *Xenopus laevis* ova. *Dev. Biol.* 40: 102-115.

WONG, A. Y.-C., 1967. Studies on mating behavior and maximum distance for copulation in the barnacles *Balanus glandula* (Darwin, 1854), *Balanus tintinnabulum* (L., 1758) and *Chthamalus dalli* (Pilsbry, 1916). Final Papers, Biol. 175h. Hopkins Marine Station. (Unpublished).

WOODIN, S. A., 1972. Polychaete abundance patterns in a marine soft-sediment environment: The importance of biological interactions. Doctoral Thesis, Univ. of Washington.

WOODS, J. H., 1969. Histochemistry and ultrastructure of oogenesis in *Ibla quadrivalvis* and *Tetraclita rosea* and the biochemical composition of developing embryos of *Ibla quadrivalvis* (Crustacea, Cirripedia). M.S. Thesis. Univ. of Sydney. (Unpublished).

WOOLLACOTT, R. M., 1974. Microfilaments and mechanism of light-triggered sperm release in ascidians. *Dev. Biol.* 40: 186-195.

WRENN, J.T. and N. K. WESSELLS, 1969. An ultrastructural study of lens invagination in the mouse. *J. Exp. Zool.* 171: 359-368.

YAMADA, C., A. J. CLARK and M. E. SWENDSEID, 1967. Actinomycin D: Effect on amino acid absorption from rat jejunal loops. *Science* 158: 129-130.

YAMADA, K. M., 1974. Cell morphogenetic movements. In: *Handbook of Teratology.* (J. G. Wilson and F. C. Fraser, eds.). New York: Plenum Publishers. (In press).

YAMADA, K. M., B. S. SPOONER and N. K. WESSELLS, 1970. Axon growth: Roles of microfilaments and microtubules. *Proc. Nat. Acad. Sci. U.S.* 66: 1206-1212.

YAMADA, K. M., B. S. SPOONER and N. K. WESSELLS, 1971. Ultrastructure and function of growth cones and axons in cultured nerve cells. *J. Cell Biol.* 49: 614-635.

YAMADA, K. M. and N. K. WESSELLS, 1973. Cytochalasin B: effects on membrane ruffling, growth cone and microspike activity, and microfilament structure not due to altered glucose transport. *Dev. Biol.* 31: 413-420.

YASUDA, T., 1970. Ecological studies on the marine fouling organisms occurring on the coast of Fukui Prefecture. Observations on the ecology of four species of barnacles found at Otomi in Uchiura Bay. *Bull. Japanese Soc. Sci. Fish.* 36: 1007.

YASUGI, R., 1937. On the swimming larvae of *Mitella mitella* (L.). *Bot. and Zool.* 5: 792-796.

ZENKEWITCH, L. A., 1935. On growth rate in different seasons of the year. *Wiss. Ber. Moskauer Staats. Univ.* 4: 135-138.

ZIGMOND, S. H. and J. G. HIRSCH, 1972a. Cytochalasin B: Inhibition of D-2 deoxyglucose transport into leukocytes and fibroblasts. *Science* 176: 1432-1434.

ZIGMOND, S. H. and J. G. HIRSCH, 1972b. Effects of cytochalasin B on polymorphonuclear leucocyte locomotion, phagocytosis and glycolysis. *Exp. Cell Res.* 73L 383-393.

ZISSLER, D. and K. SANDER, 1973. The cytoplasmic architecture of the egg cell of *Smittia* sp. (Diptera, Chironomidae). I. Anterior and posterior pole regions. *Wilhelm Roux' Archiv* 172: 175-186.

APPENDIX I

Appendix I. *Computer program calculating the total daily immersion times at specific intertidal heights, with data given for 5 months.*

```
                  PROGRAM TIDES(INPUT,OUTPUT)
000003            DIMENSION HIGH(4),TUN(4),TITLE(4,2)
000003            DIMENSION IH(3,4),IM(3,4),AP(3,4),DS(3,4),TIDE(3,4),TIME(3,4)
000003            DIMENSION MM(3,2),ID(3,2),I(7),H(7)
000003            DO 2 I=1,4
000005            DO 1 M=1,2
000006            TITLE(I,M) = 5H
000012          1 CONTINUE
000013          2 CONTINUE
            C         READ NUMBER OF HIGHTS TO PROCESS
000015            READ 3,NUMM
000023          3 FORMAT(I1)
000023            IF(NUMM.GT.4) STOP
000030            READ 6,((TITLE(I,M),M=1,2),HIGH(I),I=1,NUMM)
000051          6 FORMAT(2A5,F10.1)
000051            DO 20 I=2,3
000053            READ 10,(MM(I,K),K=1,2),ID(I,1),ID(I,2),(IH(I,J),IM(I,J),
                1 AP(I,J),DS(I,J),TIDE(I,J),J=1,4)
000120         10 FORMAT(2A5,2A1,2X,2I2,2A2,2X,F5.1,2I2,2A2,2X,F5.1,
                1                    2I2,2A2,2X,F5.1,2I2,2A2,2X,F5.1)
000120         20 CONTINUE
            C         CHECK IF LAST DATA CARD HAS BEEN READ
000122         22 IF(AP(3,2).EQ.2H  ) GO TO 999
000124            DO 40 I=1,2
000126            DO 30 J=1,4
000127            IH(I,J) = IH(I+1,J)
000134            IM(I,J) = IM(I+1,J)
000136            AP(I,J) = AP(I+1,J)
000141            DS(I,J) = DS(I+1,J)
000143            TIDE(I,J) = TIDE(I+1,J)
000145         30 CONTINUE
000147            DO 35 K=1,3
000150            MM(I,K) = MM(I+1,K)
000156         35 CONTINUE
000160            DO 38 L=1,2
000161            ID(I,L) = ID(I+1,L)
000167         38 CONTINUE
000171         40 CONTINUE
000173            I=3
000174            READ 10,(MM(I,K),K=1,2),ID(I,1),ID(I,2),(IH(I,J),IM(I,J),
                1 AP(I,J),DS(I,J),TIDE(I,J),J=1,4)
000241            DO 140 I=1,3
000243            DO 130 J=1,4
000244            AFTER = 0.0
000245            DST = 0.0
000246            IF(AP(I,J).EQ.2HAM.AND.IH(I,J).EQ.12) AFTER=-12.
000264            IF(AP(I,J).EQ.2HPM.AND.IH(I,J).NE.12) AFTER = 12.
000303            IF(DS(I,J).EQ.2HD )  DST = -1.0
000311            TIME(I,J) = IH(I,J) + IM(I,J)/60. + (I-2)*24. + AFTER + DST
```

```
000327      130 CONTINUE
000331      140 CONTINUE
000333          T(1)=TIME(1,4)  $  H(1)=TIDE(1,4)
000336          IF(T(1).GT.-12.) GO TO 145
000341          T(1)=TIME(1,3)  $  H(1)=TIDE(1,3)
000343      145 T(2)=TIME(2,1)  $  H(2)=TIDE(2,1)
000346          T(3)=TIME(2,2)  $  H(3)=TIDE(2,2)
000351          T(4)=TIME(2,3)  $  H(4)=TIDE(2,3)

000354          T(5)=TIME(2,4)  $  H(5)=TIDE(2,4)
000357          T(5)=TIME(3,1)  $  H(6)=TIDE(3,1)
000362          T(7)=TIME(3,2)  $  H(7)=TIDE(3,2)
000365          IF(ABS(T(5)).GT.0.1) GO TO 160
000372          T(5)=T(6)  $  H(5)=H(6)
000374          T(6)=T(7)  $  H(6)=H(7)
000376      160 IF(T(6).GT.24.) GO TO 170
000402          DO 165 I=1,6
000403          T(I)=T(I+1)  $  H(I)=H(I+1)
000406      165 CONTINUE
000410      170 DO 200 II=1,NUMH
000412          EL = HIGH(II)
000414          CALL UNDER(T,H,EL,TU)
000417          TUN(II) = TU
000421      200 CONTINUE
000424          IF(ID(2,1).EQ.1H .AND.ID(2,2).EQ.1H1)
           1 PRINT 210,((TITLE(I,M),M=1,2),I=1,4),(HIGH(N),N=1,NUMH)
000461      210 FORMAT(1H1//55X*HOURS SPENT SUBMERGED*////
           15X*DATE*11X*TIDE 1*10X*TIDE 2*10X*TIDE 3*10X*TIDE 4*
           2 7X,2A5,2X,2A5,2X,2A5,2X,2A5//
           3 16X*TIME*3X*HEIGHT*3X*TIME*3X*HEIGHT*
           4  3X*TIME*3X*HEIGHT*3X*TIME*3X*HEIGHT*
           5 5X,F5.1* FT*4X,F5.1* FT*4X,F5.1* FT*4X,F5.1* FT*///)
000461          IF(AP(2,4).EQ.2H  ) GO TO 274
000463          PRINT 250,(MM(2,M),M=1,2),ID(2,1),ID(2,2),(IH(2,J),IM(2,J),
           1 AP(2,J),DS(2,J),TIDE(2,J),J=1,4),(TUN(II),II=1,NUMH)
000533      250 FORMAT(1X,2A5,2A1,2X,2I2,2A2,F5.1,
           1              3X,2I2,2A2,F5.1,
           2              3X,2I2,2A2,F5.1,
           3              3X,2I2,2A2,F5.1,
           4 8X,F5.2,7X,F5.2,7X,F5.2,7X,F5.2)
000533          GO TO 300
000534      274 PRINT 275,(MM(2,M),M=1,2),ID(2,1),ID(2,2),(IH(2,J),IM(2,J),
           1 AP(2,J),DS(2,J),TIDE(2,J),J=1,3),(TUN(II),II=1,NUMH)
000604      275 FORMAT(1X,2A5,2A1,2X,2I2,2A2,F5.1,
           1                        3X,2I2,2A2,F5.1,
           2                        3X,2I2,2A2,F5.1,16X,
           3 8X,F5.2,7X,F5.2,7X,F5.2,7X,F5.2)
000604      300 GO TO 22
000605      999 STOP
000607          END
```

```
                SUBROUTINE UNDER(T,H,E,SB)
000007          DIMENSION T(7),H(7)
000007          SB = 0.0
000007          DO 900 I=1,5
000011          SA=0.0
000012          DT = T(I+1) - T(I)
000014          IF(DT.LT.0.0)  PRINT 50
000024          TL = AMAX1(0.,T(I))  $  TM = AMIN1(24.,T(I+1))
000035       50 FORMAT(*.........NEGATIVE TIME......*)
000035          IF(H(I+1)-H(I))400,100,200
           C    TIDE NOT MOVING
000041      100 IF(E.LE.H(I)) SA = TM-TL
000046          PRINT 150
000052      150 FORMAT(1H+,128X*TNM*)
000052          GO TO 800
           C   TIDE COMING IN
000055      200 IF(E.LT.H(I+1)) GO TO 210
000060          SA=0.0
000061          GO TO 800
000061      210 IF(E.GT.H(I)) GO TO 220
000065          SA=TM-TL
000067          GO TO 800
000067      220 TS=T(I+1)-DT*ACOS(2.*(E-H(I))/(H(I+1)-H(I))-1.)/3.1415927
000106          IF(TS.LE.TM) SA=TM-AMAX1(TS,TL)
000116          GO TO 800
           C    TIDE GOING OUT
000117      400 IF(E.LT.H(I)) GO TO 410
000122          SA=0.0
000123          GO TO 800
000124      410 IF(E.GT.H(I+1)) GO TO 420
000130          SA=TM-TL
000132          GO TO 800
000132      420 TS=T(I)+DT*ACOS(2.*(E-H(I+1))/(H(I)-H(I+1))-1.)/3.1415927
000151          IF(TS.GT.TL) SA=AMIN1(TS,TM)-TL
000160          GO TO 800
000161      800 SB=SB+SA
000163      900 CONTINUE
000165          RETURN
000165          END
```

Hours spent immersed

DATE		TIDE 1		TIDE 2		TIDE 3		TIDE 4		EAGLE HI	EAGLE LO	EDWARDS HI	EDWARDS LO
		TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	5.0 FT	1.0 FT	6.0 FT	3.0 FT
APRIL	1	439AM	7.3	1130AM	.2	7 3PM	7.1	1130PM	5.1	14.70	20.84	9.08	17.72
	2	456AM	7.2	12 7PM	-.1	754PM	7.1			14.43	20.10	9.60	17.12
	3	12 5AM	5.6	5 9AM	7.1	1245PM	-.2	9 1PM	7.1	13.85	19.78	10.33	16.69
	4	1250AM	6.1	457AM	7.1	130PM	-.1	1012PM	7.1	13.02	19.59	11.16	16.14
	5	137AM	6.4	5 7AM	7.1	221PM	0.0	1121PM	7.3	12.56	19.57	10.64	15.85
	6	245AM	6.6	522AM	6.9	316PM	.1			11.87	19.35	9.81	15.37
	7	1215AM	7.4	412AM	6.4	545AM	6.5	415PM	.3	11.85	19.99	9.50	15.61
	8	1250AM	7.6	555AM	5.9	955AM	5.9	515PM	.5	13.95	21.19	6.41	17.27
	9	117AM	7.7	652AM	4.9	1144AM	6.0	610PM	.9	13.60	22.85	5.34	18.34
	10	142AM	7.8	726AM	3.7	1 9PM	6.3	7 5PM	1.5	11.38	24.00	7.04	19.51
	11	2 5AM	7.9	8 9AM	2.3	220PM	6.9	754PM	2.2	11.27	24.00	8.06	17.93
	12	227AM	8.0	844AM	.9	324PM	7.5	845PM	3.1	11.64	23.00	8.92	19.16
	13	256AM	8.1	927AM	-.5	432PM	8.0	933PM	4.0	12.39	20.26	9.71	17.99
	14	321AM	8.2	10 8AM	-1.5	529PM	8.4	1022PM	4.8	13.86	19.24	10.51	17.30
	15	350AM	8.3	1051AM	-2.2	631PM	8.5	11 9PM	5.6	14.95	18.56	11.44	16.75
	16	424AM	8.3	1140AM	-2.5	733PM	8.5	1158PM	6.1	14.57	18.19	13.09	16.38
	17	457AM	8.1	1230PM	-2.3	842PM	8.4			13.22	17.12	12.13	15.17
	18	1256AM	6.5	540AM	7.7	121PM	-1.8	945PM	8.2	13.88	18.21	12.66	16.04
	19	2 4AM	6.5	621AM	7.1	220PM	-1.0	1047PM	8.2	13.67	18.75	12.23	16.17
	20	538AM	6.2	738AM	6.3	317PM	-.1	1142PM	8.1	13.76	19.91	11.84	16.70
	21	648AM	5.3	928AM	5.7	419PM	.8			14.01	21.86	6.55	17.41
	22	1224AM	8.1	723AM	4.3	1115AM	5.4	521PM	1.7	11.02	24.00	6.05	19.24
	23	1259AM	8.0	8 0AM	3.3	1252PM	5.5	624PM	2.5	10.39	24.00	5.57	21.13
	24	125AM	7.9	827AM	2.4	2 7PM	5.9	713PM	3.2	10.90	24.00	5.22	20.96
	25	153AM	7.8	850AM	1.5	3 9PM	6.4	754PM	3.9	11.74	24.00	7.50	19.39
	26	215AM	7.7	911AM	.8	4 4PM	6.9	838PM	4.6	13.07	22.45	8.69	18.53
	27	235AM	7.5	931AM	.1	453PM	7.3	918PM	5.1	15.25	20.73	9.44	17.79
	28	257AM	7.4	955AM	-.4	542PM	7.6	10 1PM	5.6	14.86	19.92	10.51	17.28
	29	318AM	7.3	1024AM	-.8	623PM	7.7	1036PM	6.0	14.50	19.35	13.14	16.87
	30	429AMD	7.2	1159AMD	-1.0	8 6PMD	7.8			14.08	18.97	12.68	16.49

Hours spent immersed

DATE		TIDE 1		TIDE 2		TIDE 3		TIDE 4		EAGLE HI	EAGLE LO	EDWARDS HI	EDWARDS LO
		TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	5.0 FT	1.0 FT	6.0 FT	3.0 FT
MAY	1	1215AMD	6.3	437AMD	7.2	1233PMD	-1.1	9 0PMD	7.8	13.55	18.61	12.10	16.05
	2	1257AMD	6.5	430AMD	7.2	113PMD	-1.0	950PMD	7.9	12.97	18.37	11.43	15.63
	3	149AMD	6.7	439AMD	7.1	155PMD	-.8	1038PMD	7.9	12.63	18.44	10.97	15.47
	4	249AMD	6.6	5 6AMD	6.9	242PMD	-.5	1121PMD	8.0	12.50	18.76	10.72	15.53
	5	4 6AMD	6.3	541AMD	6.4	330PMD	0.0	1156PMD	8.0	12.33	19.52	10.14	15.75
	6	520AMD	6.0	615AMD	6.8	427PMD	.6			12.17	21.01	8.54	16.15
	7	1231AMD	8.0	637AMD	4.6	11 9AMD	5.2	522PMD	1.4	10.64	24.00	5.86	18.76
	8	1254AMD	8.1	718AMD	3.3	1255PMD	5.5	617PMD	2.3	10.10	23.90	5.29	20.64
	9	122AMD	8.1	755AMD	1.8	226PMD	6.1	719PMD	3.3	10.99	24.00	6.38	19.81
	10	149AMD	8.2	836AMD	.2	341PMD	7.0	817PMD	4.3	12.45	21.03	8.87	18.12
	11	220AMD	8.3	919AMD	-1.2	445PMD	7.8	917PMD	5.2	15.19	19.32	10.52	17.21
	12	249AMD	8.4	10 1AMD	-2.3	547PMD	8.5	10 6PMD	5.9	14.77	18.41	12.57	16.58
	13	324AMD	8.5	1048AMD	-3.0	644PMD	8.9	11 3PMD	6.4	14.50	17.90	13.56	16.21
	14	359AMD	8.5	1134AMD	-3.3	739PMD	9.0	1157PMD	6.7	14.24	17.62	13.30	15.94
	15	442AMD	8.2	1220PMD	-3.1	833PMD	9.0			14.08	17.61	13.10	15.86
	16	1255AMD	6.8	524AMD	7.8	1 7PMD	-2.5	924PMD	8.9	13.92	17.78	12.84	15.86
	17	2 8AMD	6.7	612AMD	7.2	157PMD	-1.7	1017PMD	8.7	13.87	18.29	12.61	16.08
	18	4 2AMD	6.2	715AMD	6.3	248PMD	-.6	11 0PMD	8.6	13.94	19.34	12.19	16.59
	19	620AMD	5.2	849AMD	5.5	332PMD	.5	1140PMD	8.4	14.18	21.29	7.33	17.51
	20	7 9AMD	4.2	1034AMD	4.9	426PMD	1.7			8.63	24.00	6.61	19.12
	21	1218AMD	8.3	748AMD	3.1	1229PMD	4.8	515PMD	2.8	8.07	24.00	6.23	22.08
	22	1250AMD	8.1	820AMD	2.1	2 6PMD	5.3	611PMD	3.9	10.26	23.83	5.92	19.88
	23	115AMD	8.0	842AMD	1.2	327PMD	6.0	7 6PMD	4.8	13.22	24.00	6.02	18.60
	24	138AMD	7.8	9 7AMD	.4	424PMD	6.6	8 3PMD	5.5	14.86	21.16	9.55	17.71
	25	2 2AMD	7.7	931AMD	-.3	512PMD	7.2	858PMD	6.0	14.59	19.92	13.13	17.13
	26	229AMD	7.6	10 0AMD	-.9	6 1PMD	7.7	947PMD	6.4	14.31	19.15	12.97	16.67
	27	252AMD	7.5	1029AMD	-1.3	644PMD	8.0	1035PMD	6.7	14.03	18.66	12.73	16.32
	28	312AMD	7.5	11 1AMD	-1.5	726PMD	8.2	1118PMD	6.9	13.81	18.38	12.52	16.08
	29	327AMD	7.4	1137AMD	-1.7	8 8PMD	8.4			13.48	18.06	12.17	15.76
	30	12 6AMD	7.0	322AMD	7.4	1212PMD	-1.7	843PMD	8.4	13.04	17.81	11.68	15.42
	31	1 0AMD	6.9	331AMD	7.2	1247PMD	-1.5	924PMD	8.5	12.70	17.79	11.23	15.23

Hours spent immersed

DATE		TIDE 1		TIDE 2		TIDE 3		TIDE 4		EAGLE HI	EAGLE LO	EDWARDS HI	EDWARDS LO
		TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	5.0 FT	1.0 FT	6.0 FT	3.0 FT
JUNE	1	149AMD	6.7	4 6AMD	6.9	129PMD	-1.2	951PMD	8.5	12.70	18.08	11.12	15.37
	2	256AMD	6.3	447AMD	6.4	211PMD	-.6	1022PMD	8.5	12.68	18.76	10.74	15.67
	3	4 0AMD	5.5	537AMD	5.6	253PMD	.2	1049PMD	8.5	12.37	20.07	6.61	16.13
	4	456AMD	4.4	929AMD	5.0	340PMD	1.3	1115PMD	8.4	7.84	24.00	6.05	18.60
	5	549AMD	3.1	1125AMD	4.9	435PMD	2.5	1144PMD	8.5	7.36	24.00	5.80	21.11
	6	639AMD	1.6	113PMD	5.4	532PMD	3.8			10.26	24.00	5.85	19.22
	7	1212AMD	8.5	722AMD	.1	249PMD	6.3	634PMD	5.0	14.84	20.62	8.32	17.56
	8	1247AMD	8.6	810AMD	-1.3	358PMD	7.3	738PMD	6.0	14.34	18.72	13.11	16.50
	9	122AMD	8.7	859AMD	-2.4	5 1PMD	8.1	845PMD	6.7	14.27	18.06	13.22	16.17
	10	2 3AMD	8.7	942AMD	-3.1	556PMD	8.7	947PMD	7.0	14.13	17.63	13.16	15.89
	11	245AMD	8.7	1031AMD	-3.4	645PMD	9.0	1050PMD	7.2	14.06	17.45	13.13	15.77
	12	327AMD	8.4	1118AMD	-3.3	733PMD	9.2	1156PMD	7.0	13.99	17.45	13.04	15.74
	13	416AMD	8.0	12 4PMD	-2.9	816PMD	9.1			14.00	17.65	12.98	15.84
	14	1 0AMD	6.7	512AMD	7.4	1245PMD	-2.1	856PMD	9.0	14.15	18.18	13.01	16.17
	15	216AMD	6.1	614AMD	6.7	129PMD	-1.1	935PMD	8.9	14.37	19.03	12.99	16.67
	16	4 6AMD	5.3	726AMD	5.8	2 9PMD	0.0	10 7PMD	8.7	14.55	20.41	7.09	17.38
	17	515AMD	4.3	852AMD	5.1	252PMD	1.3	1036PMD	8.5	10.04	24.00	6.71	18.70
	18	6 4AMD	3.3	1036AMD	4.6	333PMD	2.6	11 7PMD	8.3	8.22	24.00	6.44	21.25
	19	649AMD	2.3	1230PMD	4.8	415PMD	3.8	1135PMD	8.1	8.36	24.00	6.42	20.27
	20	721AMD	1.4	213PMD	5.4	5 4PMD	4.8			12.24	24.00	6.54	18.48
	21	12 1AMD	8.0	753AMD	.5	329PMD	6.1	6 7PMD	5.7	14.18	21.22	8.57	17.38
	22	1230AMD	7.8	825AMD	-.2	432PMD	6.9	7 9PMD	6.4	13.47	19.31	11.84	16.25
	23	1 3AMD	7.7	9 0AMD	-.8	513PMD	7.4	818PMD	6.8	13.85	19.03	12.41	16.38
	24	129AMD	7.7	929AMD	-1.3	556PMD	7.9	921PMD	7.1	13.71	18.48	12.38	16.06
	25	2 4AMD	7.6	10 4AMD	-1.6	637PMD	8.2	1014PMD	7.1	13.62	18.20	12.33	15.89
	26	233AMD	7.6	1040AMD	-1.8	711PMD	8.4	11 3PMD	7.1	13.57	18.03	12.31	15.79
	27	251AMD	7.5	1117AMD	-1.9	742PMD	8.5	1152PMD	6.9	13.38	17.86	12.10	15.62
	28	330AMD	7.3	1150AMD	-1.8	8 9PMD	8.6			13.47	17.99	12.18	15.73
	29	1246AMD	6.5	413AMD	6.9	1228PMD	-1.5	834PMD	8.6	13.32	18.05	11.80	15.69
	30	130AMD	5.9	521AMD	6.5	1 2PMD	-.9	857PMD	8.6	14.03	19.06	10.49	16.52

Hours spent immersed

DATE		TIDE 1		TIDE 2		TIDE 3		TIDE 4		EAGLE HI	EAGLE LO	EDWARDS HI	EDWARDS LO
		TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	5.0 FT	1.0 FT	6.0 FT	3.0 FT
JULY	1	224AMD	5.1	648AMD	5.9	143PMD	0.0	920PMD	8.6	14.65	20.44	6.13	17.44
	2	321AMD	4.1	815AMD	5.3	226PMD	1.1	946PMD	8.6	10.08	24.00	5.93	18.63
	3	410AMD	2.8	955AMD	5.0	3 9PMD	2.4	1014PMD	8.6	7.14	24.00	5.94	19.02
	4	5 4AMD	1.5	1145AMD	5.2	357PMD	3.8	1042PMD	8.6	9.52	24.00	5.92	18.99
	5	559AMD	.2	135PMD	5.8	452PMD	5.1	1115PMD	8.6	14.30	20.67	6.41	17.35
	6	656AMD	-1.0	3 3PMD	6.7	555PMD	6.1	1153PMD	8.7	13.95	18.92	12.46	16.41
	7	746AMD	-1.9	412PMD	7.6	7 8PMD	6.8			13.81	18.09	12.61	15.95
	8	1242AMD	8.6	839AMD	-2.6	5 7PMD	8.2	829PMD	7.2	13.44	17.33	12.35	15.39
	9	134AMD	8.5	931AMD	-3.0	550PMD	8.6	940PMD	7.1	13.82	17.48	12.80	15.67
	10	233AMD	8.3	1020AMD	-3.0	632PMD	8.8	1050PMD	6.8	14.00	17.60	12.99	15.81
	11	325AMD	8.0	11 3AMD	-2.6	7 7PMD	8.9	1151PMD	6.3	14.22	17.93	13.19	16.08
	12	425AMD	7.5	1145AMD	-2.0	742PMD	8.8			14.45	18.40	12.10	16.42
	13	1248AMD	5.7	521AMD	6.9	1226PMD	-1.1	811PMD	8.7	14.53	19.00	10.91	16.73
	14	150AMD	4.9	625AMD	6.3	1 3PMD	-.1	838PMD	8.5	13.57	20.43	8.35	17.64
	15	239AMD	4.1	734AMD	5.6	130PMD	1.1	9 4PMD	8.3	11.05	24.00	5.97	18.73
	16	333AMD	3.3	851AMD	5.1	212PMD	2.2	932PMD	8.1	8.69	24.00	5.87	20.36
	17	424AMD	2.5	1026AMD	4.9	247PMD	3.4	955PMD	7.9	7.52	24.00	5.93	20.85
	18	512AMD	1.7	1219PMD	5.1	330PMD	4.5	1023PMD	7.7	9.76	24.00	6.17	18.77
	19	6 5AMD	1.0	2 9PMD	5.6	411PMD	5.4	1054PMD	7.6	13.50	24.00	6.47	17.47
	20	657AMD	.3	318PMD	6.3	520PMD	6.2	1127PMD	7.5	13.45	20.53	11.30	16.74
	21	738AMD	-.2	412PMD	6.9	630PMD	6.7			13.37	19.58	11.62	16.34
	22	12 9AMD	7.5	821AMD	-.8	655PMD	7.4	749PMD	6.9	13.37	18.81	11.84	16.03
	23	1252AMD	7.5	9 2AMD	-1.2	530PMD	7.8	9 1PMD	6.9	13.36	18.34	11.96	15.82
	24	141AMD	7.6	939AMD	-1.5	6 3PMD	8.0	10 3PMD	6.8	13.59	18.29	12.25	15.93
	25	234AMD	7.4	1015AMD	-1.7	630PMD	8.2	1045PMD	6.4	13.88	18.30	12.62	16.09
	26	323AMD	7.3	1053AMD	-1.6	653PMD	8.3	1132PMD	5.8	14.18	18.53	10.89	16.35
	27	412AMD	7.1	1127AMD	-1.3	714PMD	8.3			14.49	18.92	10.67	16.69
	28	1215AMD	5.1	515AMD	6.8	12 7PMD	-.7	733PMD	8.3	13.41	19.70	9.29	17.30
	29	1258AMD	4.2	617AMD	6.4	1243PMD	.1	755PMD	8.3	11.76	20.83	7.83	17.99
	30	145AMD	3.2	723AMD	6.1	119PMD	1.2	818PMD	8.3	10.83	24.00	6.51	19.19
	31	233AMD	2.1	843AMD	5.7	2 0PMD	2.4	843PMD	8.3	10.15	24.00	5.42	17.52

Hours spent immersed

DATE		TIDE 1		TIDE 2		TIDE 3		TIDE 4		EAGLE HI	EAGLE LO	EDWARDS HI	EDWARDS LO
		TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	TIME	HEIGHT	5.0 FT	1.0 FT	6.0 FT	3.0 FT
AUGUST	1	324AMD	1.0	1010AMD	5.6	243PMD	3.7	912PMD	8.3	10.65	24.00	5.81	18.55
	2	425AMD	.1	1159AMD	5.9	332PMD	4.9	947PMD	8.3	13.48	20.50	6.40	17.29
	3	522AMD	-.7	143PMD	6.4	433PMD	5.9	1028PMD	8.3	13.73	19.11	10.95	16.35
	4	625AMD	-1.3	3 4PMD	7.1	543PMD	6.6	1121PMD	8.2	13.45	18.38	12.03	15.90
	5	727AMD	-1.8	4 0PMD	7.7	712PMD	6.9			13.51	18.00	12.24	15.75
	6	1223AMD	8.0	827AMD	-2.0	443PMD	8.0	839PMD	6.7	13.08	17.36	11.87	15.22
	7	134AMD	7.8	919AMD	-2.0	518PMD	8.3	956PMD	6.2	14.11	18.21	12.95	16.16
	8	242AMD	7.6	10 8AMD	-1.8	553PMD	8.3	1051PMD	5.5	14.44	18.53	10.18	16.48
	9	344AMD	7.3	1050AMD	-1.2	622PMD	8.3	1140PMD	4.8	13.07	19.18	10.21	16.99
	10	442AMD	7.0	1125AMD	-.5	647PMD	8.2			12.78	20.01	9.27	17.56
	11	1216AMD	4.0	542AMD	6.6	12 1PMD	.4	710PMD	8.0	11.76	21.43	8.07	18.32
	12	1257AMD	3.3	642AMD	6.2	1236PMD	1.4	736PMD	7.8	10.90	23.95	6.65	18.85
	13	137AMD	2.6	742AMD	5.9	1 4PMD	2.4	755PMD	7.6	10.44	24.00	4.87	18.60
	14	221AMD	2.0	849AMD	5.6	141PMD	3.4	819PMD	7.4	10.14	24.00	4.85	20.16
	15	3 3AMD	1.5	10 9AMD	5.5	216PMD	4.3	842PMD	7.3	10.83	24.00	5.11	18.97
	16	352AMD	1.1	1157AMD	5.6	257PMD	5.1	9 9PMD	7.2	14.14	24.00	5.54	17.68
	17	447AMD	.7	136PMD	6.0	346PMD	5.8	938PMD	7.1	13.65	21.60	6.53	16.87
	18	549AMD	.4	245PMD	6.5	440PMD	6.3	10 8PMD	7.0	13.20	20.60	11.17	16.45
	19	647AMD	0.0	337PMD	7.0	617PMD	6.6	1127PMD	6.9	12.79	19.69	11.56	16.06
	20	739AMD	-.4	414PMD	7.3	746PMD	6.5			13.17	19.23	11.40	16.11
	21	1239AMD	6.9	829AMD	-.7	441PMD	7.6	854PMD	6.2	13.33	18.74	11.74	15.98
	22	140AMD	7.0	9 9AMD	-.9	5 4PMD	7.8	943PMD	5.6	14.15	19.11	9.64	16.59
	23	239AMD	7.0	949AMD	-.8	527PMD	7.8	1019PMD	4.9	13.27	19.40	8.46	16.94
	24	339AMD	7.1	1024AMD	-.5	545PMD	7.9	11 1PMD	4.0	11.45	19.98	9.24	17.51
	25	436AMD	7.1	11 5AMD	0.0	6 5PMD	7.9	1142PMD	2.9	11.81	20.79	8.60	17.02
	26	537AMD	7.0	1142AMD	.8	625PMD	8.0			11.17	22.58	8.21	16.67
	27	1222AMD	1.8	638AMD	6.9	1217PMD	1.8	649PMD	8.0	10.99	22.81	8.03	16.66
	28	1 7AMD	.8	745AMD	6.8	1 0PMD	3.0	715PMD	8.0	11.31	22.50	8.12	19.27
	29	156AMD	0.0	9 0AMD	6.6	141PMD	4.1	741PMD	8.0	12.05	21.08	8.25	18.38
	30	251AMD	-.6	1025AMD	6.6	230PMD	5.1	815PMD	8.0	15.16	19.79	9.14	17.67
	31	346AMD	-.9	12 1PMD	6.7	326PMD	5.9	857PMD	7.8	14.54	18.95	11.32	17.04

Figure 62. *Pollicipes polymerus*. Total daily immersion times during the reproductive period (1972) Edward's Reef for the high and low intertidal populations. Percent brooding is given for the populations at this locality.

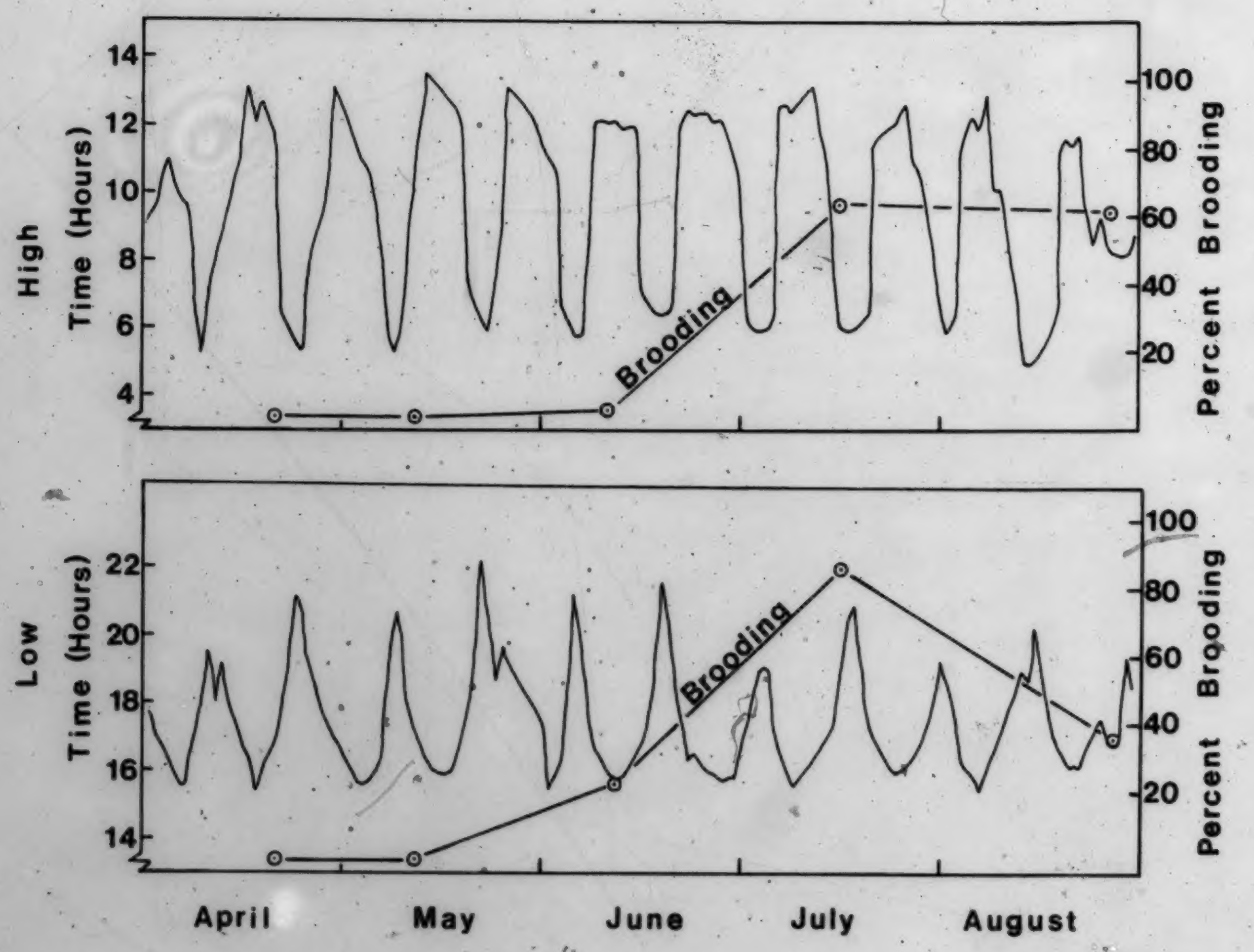
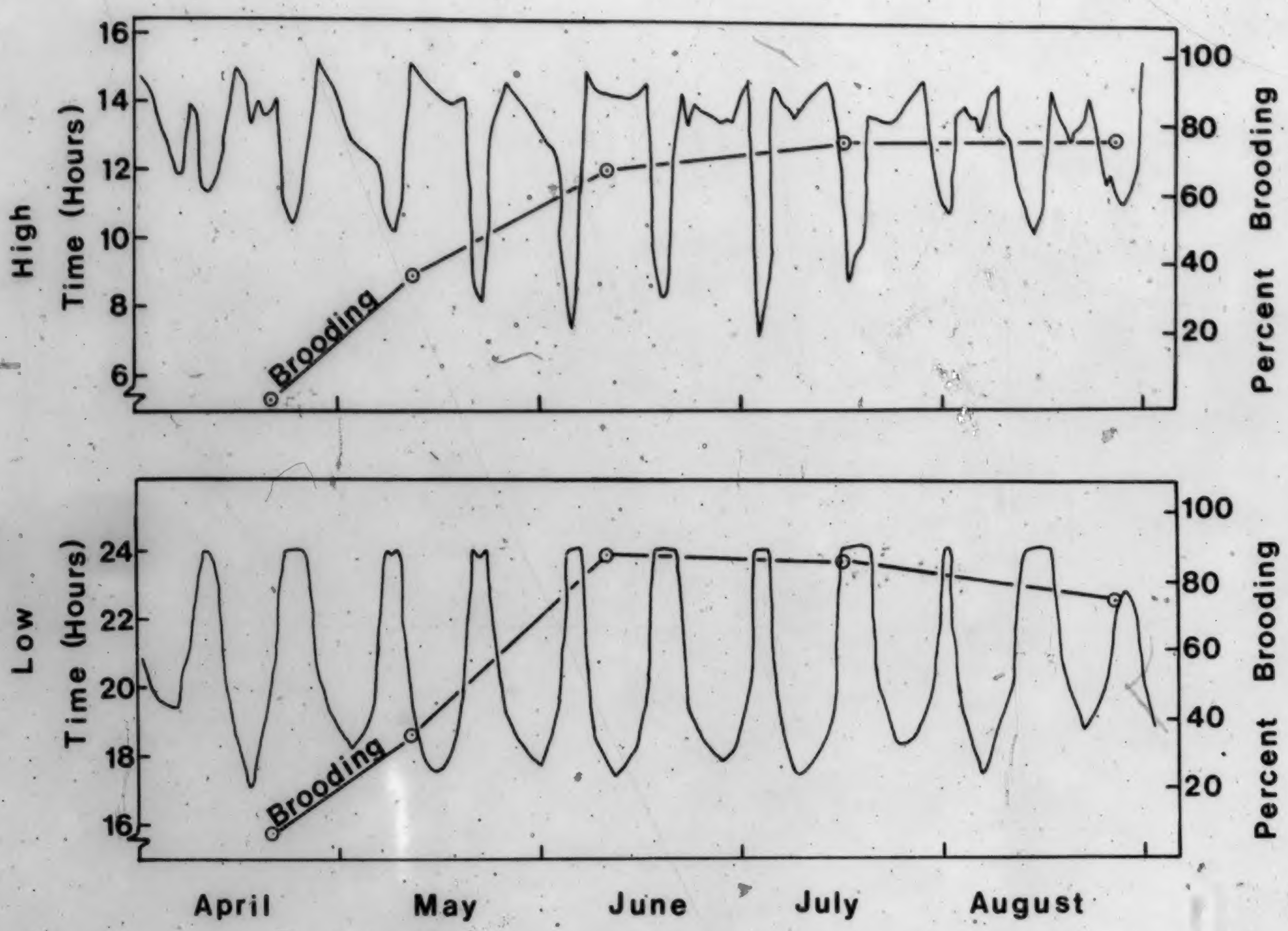

High
Time (Hours)
14
12
10
8
6
4
Percent Brooding
100
80
60
40
20
Brooding
Low
Time (Hours)
22
20
18
16
14
Percent Brooding
100
80
60
40
20
Brooding
April
May
June
July
August

Figure 63. *Pollicipes polymerus*. Total daily immersion times during the reproductive period (1972) at Eagle Point for the high and low intertidal populations. Percent brooding is given for the populations at this locality.


High
Time (Hours)
16
14
12
10
8
6
Brooding
Percent Brooding
100
80
60
40
20
Low
Time (Hours)
24
22
20
18
16
Brooding
Percent Brooding
100
80
60
40
20
April
May
June
July
August